Impact of a (poly)phenol-rich extract from the brown algae *Ascophyllum nodosum* on DNA damage and antioxidant activity in an overweight/obese population: a randomised controlled trial

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Running head: Ascophyllum nodosum (poly)phenols impact DNA damage in the obese.

Abbreviations used: SPE, seaweed (poly)phenol extract; CRP, C-reactive protein; CVD, cardiovascular disease; ROS, reactive oxygen species; TNF-α, tumour necrocis factor alpha; COX, cyclooxygenase; UUREC, University of Ulster Research Ethics Committee; WISP, Weighed Intake Software Program; TF, tissue factor; TOC, Total oxidative capacity; TMB, tetramethylbenzidine; IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; UHPLC-HRMS: ultra-high performance liquid chromatography-high resolution-mass spectrometry; VIP: variable of importance in projection; OPLS-DA: Orthogonal Partial Least Square Discriminant Analysis.

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ABSTRACT

Background: Epidemiological evidence suggests a diet rich in (poly)phenols has beneficial effects on many chronic diseases. Brown seaweed is a rich source of (poly)phenols

Objective: The aim of this study was to investigate the bioavailability and effect of a brown seaweed (*Ascophyllum nodosum*) (poly)phenol extract from on DNA damage, oxidative stress, and inflammation *in vivo*.

Design: A randomised double-blind placebo-controlled crossover trial was conducted in 80 participants aged 30-65 years with a BMI $\geq$25kg/m^2. The participants consumed either a 400 mg capsule containing 100 mg of seaweed (poly)phenol and 300 mg maltodextrin or a 400 mg maltodextrin placebo control capsule daily for an 8-week period. Bioactivity was assessed with a panel of blood-based markers including lymphocyte DNA damage, plasma oxidant capacity, C-reactive protein and inflammatory cytokines. To explore the bioavailability of seaweed phenolics, an untargeted metabolomics analysis of urine and plasma samples following seaweed consumption was determined by UHPLC-HR-MS.

Results: Consumption of the seaweed (poly)phenols resulted in a modest decrease DNA damage but only in a subset of the total population who were obese. There were no significant changes in CRP, antioxidant status, inflammatory cytokines. We identified phlorotannin metabolites that are considered potential biomarkers of seaweed consumption including pyrogallol/phloroglucinol-sulfate, hydroxytrifurahol A-glucuronide, dioxinodehydroeckol-glucuronide, diphlorethol sulfates, C-O-C dimers of phloroglucinol sulfate, C-O-C dimers of phloroglucinol and diphlorethol sulfate.

Conclusion: To the best of our knowledge, this work represents the first comprehensive study investigating the bioactivity and bioavailability of seaweed (poly)phenolics in human participants. We identified several potential biomarkers of seaweed consumption. Intriguingly, the modest improvements in DNA damage were observed only in the obese subset of the total

population, the subgroup analysis should be considered exploratory as it was not preplanned; therefore, are not powered adequately. Elucidation of the biology underpinning this observation will require participant stratification according to weight in future studies.

Key words: Seaweed, Phenolic compounds, Inflammation, DNA damage, Oxidative stress, bioavailability

INTRODUCTION

Diets rich in plant-derived foods may reduce risk of chronic degenerative diseases, including cardiovascular disease (CVD), with the beneficial effects being attributable in part to highly bioactive (poly)phenolic compounds contained therein (1-3). Fruits and vegetables are well-known sources of (poly)phenols, but a less well-known source is Ascophyllum nodosum, a brown algae common to the British Isles that is rich in (poly)phenolic compounds, including, uniquely, phlorotannin (4, 5). Phlorotannins are oligomers of phloroglucinol whose concentration in seaweed is affected by numerous factors including plant size and age, water salinity, nutrient and heavy metal content, in addition to light intensity changes (6-8). *Ascophyllum nodosum* is of interest as it is one of only a few commercially sustainable seaweed species.

Phlorotannins – and brown seaweed extracts in general – exhibit beneficial effects on a range of biological processes including modulation of inflammation, reduction of oxidative stress and improvements in cardiovascular function (9-11). However, the evidence base relies heavily on cell line and small animal models, with few studies to date involving humans (12-13). Phlorotannin-rich extracts from brown seaweeds have been shown to be effective in controlling inflammation *via* a number of pathways including inhibition of pro-inflammatory cytokines including tumour necrosis factor (TNF)-α and interleukins (IL)-1β and IL-6 *in vitro* (14). The efficacy of *Ascophyllum nodosum* and other Fucoid species extracts in mitigating the effects of oxidative stress by inhibiting the generation of reactive oxygen species (ROS), preventing DNA damage and in stimulating the production of glutathione in affected cells has been demonstrated in our work and that of other researchers (15-20). Our initial *in vitro* (15) and acute *in vivo* (20) observations of the antioxidant and anti-inflammatory activities of *Ascophyllum nodosum* extract(s) gave rise to the hypothesis that longer term consumption of *Ascophyllum nodosum*-derived (poly)phenols would be of benefit *in vivo.*

A few investigations have evaluated – either *in vitro* or *in vivo* – the bioactivity of phlorotannin rich extracts from Fucoid species. For example, safe consumption levels for a (poly)phenol-enriched extract of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus* and its effects on glycaemic response have been determined in clinical trials (21). To the best of our knowledge, we now report the first clinical study aimed at specifically addressing the effects of a phlorotannin-rich extract from *Ascophyllum nodosum* on oxidative damage to DNA, plasma antioxidant capacity, inflammatory responses and chronic, low level inflammation *in vivo.*

PARTICIPANTS AND METHODS

Seaweed material

Fresh *Ascophyllum nodosum* was supplied by The Hebridean Seaweed Company, Isle of Lewis, Scotland, UK, in March 2011. The seaweed biomass was harvested by hand to ensure quality, cleaned of contaminating sand and fouling organisms and then shipped refrigerated to the processing facility (CEVA) in France where it was immediately chopped and frozen.

Preparation of food-grade seaweed extracts and capsule

A (poly)phenol-rich seaweed extract from *Ascophyllum nodosum* was produced by CEVA (France) using a food–grade solvent (ethanol:water, 60:40 vol/vol) extraction system that was specifically developed for use with fresh or frozen *Ascophyllum nodosum*. Approximately half of the produced extract was then fractionated using tangential flow ultra-filtration to produce further extracts of varying molecular weight ranges and with varying (poly)phenol content. A standardised blended (poly)phenol-rich *Ascophyllum nodosum* extract was formulated by CEVA (**Table 1**) using 175 mg of extract and 50 mg of high molecular weight fraction (>10 kDa cut off) for use in the current study, in order to maximise the seaweed (poly)phenol content

(>100 mg per day) available from the extraction of fresh or frozen *Ascophyllum nodosum* against the need to minimise the level of iodine to within accepted regulatory guidelines (<500 µg per day) (**Table 1**), heavy metal contamination was also assessed. Maltodextrin (175 mg) was added to the capsule formulation as an excipient. Blending was carried out at the food–grade CEVA facilities in France. Samples of 400 mg of the *Ascophyllum* (poly)phenol–rich blend (SPE) or a placebo containing 400 mg maltodextrin only were packed into identical white, opaque, vegetarian capsules by Irish Seaweeds, Belfast, UK and identically sized and matched capsules were used for the clinical study. The food–grade seaweed capsule was characterized by NP-HPLC and LC-MS analysis and is reported elsewhere (20) (see **Supplemental figures 1 & 2**). Phlorotannins were quantified using the Folin-Ciocalteau Method (22) using phloroglucinol as the standard. In brief, 1 mL of suitability diluted sample was reacted with 1 mL of 40 % Folin Ciocalteu reagent for 5 min, and then made alkaline with the addition of 1 mL of 100 g/L Na_2CO_3. Absorbance was read at 730 nm after the solution had developed for 1 h at room temperature. Phloroglucinol dihydrate (range 0-30 mg/L) was used as a standard and was treated in the same way as samples.

Ethics and participants

Ethical approval was received from the Ulster University Research Ethics Committee (UUREC). All participants gave written informed consent. Participants were recruited between May 2011 and August 2011 from Ulster University and the surrounding area. The intervention study ran between August 2011 and February 2012. The study was registered at clinicaltrials.gov as NCT02295878.

The study was conducted in 80 participants (age range 30-65 years). All participants were apparently healthy, non-smoking, BMI≥25 kg/m^2, omnivores, who did not habitually use vitamin or mineral supplements, as determined using a pre-screening health and lifestyle

questionnaire. Pregnant and lactating women, vegetarians and vegans and lactose–intolerant individuals were excluded from the study, as were those with chronic medical complications such as diabetes, cardiovascular disease, autoimmune/inflammatory disorders, or who had chronic medication use including anti-inflammatory agents.

Study design

The study was a 24-week randomised, double-blind, placebo-controlled crossover trial. After obtaining consent, participants were randomly assigned, in blocks of four using a random-number generator (www.randomization.com), to either the intervention or the control. In total, eighty participants were randomised to 2 groups of 40, each starting on either a 400 mg seaweed (poly)phenol extract (SPE) capsule containing 100mg of (poly)phenols or a 400 mg maltodextrin placebo control capsule (Avebe MD14P) daily for an 8-week period. The participants were supplied with all capsules in weekly labelled capsule boxes at the beginning of each phase, which was interspersed by an 8-week washout phase. During the washout phase, the participants were asked to maintain their habitual diet. Participants were asked to bring any unconsumed capsules to their study appointment at the end of each treatment phase and were also contacted weekly by the study researcher to encourage compliance and to discuss any difficulties they were experiencing.

Blood and urine sample collection

Fasting blood samples were collected before and after each phase (week 0, week 8, week 16, and week 24) by venepuncture into EDTA, serum or sodium heparin-containing tubes, as required. All blood samples were processed on ice. Lymphocytes were isolated by using Histopaque-1077, according to the manufacturer's instructions (Sigma Diagnostics, St Louis, MO), and plasma samples were prepared by centrifugation at 1000 × g for 10 min at 4 °C.

Serum samples were allowed to clot for 30 min at room temperature and then were centrifuged at 2000 × g for 10 min at 4 °C. Whole blood from sodium-heparin treated tubes was prepared according to the manufacturer's instructions (BD Bioscience Fast Immune Cytokine System). Whole blood, plasma, serum and urine samples were immediately stored at -80 °C, whereas lymphocytes were stored frozen in liquid nitrogen. All biological measurements were carried out at the end of the intervention in batches containing equal numbers of active and control phase samples in each batch, and the researchers were blinded to these samples during analyses. A 24 h urine collection occurred at each time point; volume and pH were measured and the sample mixed following which 2 × 14ml aliquots were removed and centrifuged at 1000 x g for 10 min at 4 °C. Supernatants were stored at -80 °C until required.

Questionnaire assessments

All participants completed a health and lifestyle questionnaire assessing their alcohol intake and physical activity levels, as well as a validated 4-day food diary at the mid-point during each treatment phase (active/placebo) of the study. Data on type of food and corresponding weight was entered into a food analysis database (WISP, Weighed Intake Software Program; Tinuviel Software, Warrington, U.K.) by two independent researchers and the dietary composition calculated.

DNA damage in peripheral blood mononuclear cells

Peripheral blood lymphocytes, previously isolated and stored in liquid nitrogen, were thawed and screened for basal single strand breaks (SBs) in DNA using the single cell gel electrophoresis (Comet) assay (23) as adapted by Gill *et al.* (24). Spontaneous DNA SBs are associated with an altered cell function and are considered appropriate for the substantiation of health claims in the context of protection against generic DNA damage (25). In addition,

206 resistance to induced DNA damage (SB) was measured in lymphocytes subjected to increased
207 oxidative insult *ex vivo* by pre-treating lymphocytes with 150 µmol H_2O_2/L for 5 min at 4°C,
208 before the measurement of SBs. The mean (percentage DNA in tail) was calculated from 50
209 cells per gel (each sample in triplicate) and the mean of each dataset was used in the statistical
210 analysis.
211
212 **Plasma total oxidative capacity**
213 Total oxidative capacity (TOC) measures total peroxide levels in plasma, by the reaction of
214 endogenous peroxides with peroxidases, using tetramethylbenzidine (TMB) as the
215 chromogenic substrate (26) with spectrophotometric measurement at 450 nm. To 10 µL of
216 standard (freshly-prepared hydrogen peroxide, 0.1 mmol/L) or samples in an uncoated
217 microtiter plate was added 200 µL of reaction mixture (0.05M phosphate-citrate buffer, pH 5.0;
218 TMB solution (1 mg/mL), and peroxidase (>2500U/mL) in a proportion of 100:10:1. Plates
219 were incubated at room temperature for 15 min, following which 50 µL of stop solution (2M
220 H_2SO_4) was added to all wells and absorbance at 450 nm was measured using a microplate
221 reader (GENIOS Tecan). Hydrogen peroxide standard solutions (range 0 - 1 mmol/L) were
222 freshly prepared before use.
223
224 **Lipid profile and serum C-reactive protein**
225 Plasma total cholesterol, HDL cholesterol and triglycerides were measured on an Instrument
226 Laboratory (ILAB) 600 (Warrington, UK) autoanalyzer using commercial kits (Roche
227 diagnostics, Lewis, UK) according to kit manufacturer's protocols. Plasma LDL cholesterol
228 was calculated using the Friedewald formula (27).

C-reactive protein was determined on an ILAB 600 autoanalyser using a *Quantex* CRP Ultra-Sensitive commercial kit (0.4-18.3 µg/dL) in accordance with the manufacturer's instructions.

Measurement of inflammatory markers

Intracellular cytokine levels in lymphocyte and monocyte populations and tissue factor (TF) expression were assessed using a whole blood labelling method that utilises flow-cytometry (Fast Immune Cytokine System, BD Biosciences) in accordance with manufacturer's instructions for all participants at all time-points. The method was used to measure intracellular IL-1β, IL-2, IL-6, IL-10, IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α expression in mononuclear cells. Briefly, whole blood was incubated with either lipopolysaccharide or phorbol 12-myristate 13-acetate to activate monocytes and lymphocytes, respectively. Cells were labelled with the appropriate cell surface antibody and cytokine-specific antibody and analysed on a Gallios flow cytometer (Beckman Coulter). The number and percentage of each cell type expressing the cytokine, as well as the mean channel fluorescence was recorded. The cytokine profiles were examined by ratio of TNF-α to IL-10, IL-1β to IL-10, IL-6 to IL-10 and CRP to IL-10, according to Laird *et al*. (29).

Extraction of (poly)phenols from urine and blood samples.

Urine samples were defrosted, vortexed, centrifuged at 16,110 × g for 10 min at 5 °C, and passed through 0.45 µm filter discs prior to the analysis of 50 µL aliquots by UHPLC-HR-MS. The extraction of metabolites from the plasma samples was carried out as described previously (28). Briefly, plasma samples were defrosted, vortexed and 400 µL aliquots mixed with 10 µL of ascorbic acid (10%, v/v), and 980 µL of 1% formic acid in acetonitrile. One µg of rutin was added to the samples as internal standard for plasma extraction efficiency. The samples were

254 then vortexed for 1 min and ultrasonicated for 10 min. After centrifugation at 16,110 g for 15
255 min, supernatants were reduced to dryness in vacuo using a concentrator plus (Eppendorf,
256 Hamburg, Germany) and resuspended in 150 µL of distilled water containing 1% formic acid
257 and 50 µL of methanol, which was then centrifuged at 16,110 g for 10 min. Analysis of 10 µL
258 aliquots of the supernatant was by UHPLC-HR-MS. Recovery of the internal standard was 79
259 ± 16% (n=78).

260

261 **Non-targeted analysis of urine and plasma by UHPLC-HR-MS.**

262 Plasma and urine samples were analysed using a Dionex Ultimate 3000 RP UHPLC system
263 comprising of a UHPLC pump, a PDA detector scanning from 200 to 600 nm, and an
264 autosampler operating at 4 °C (Thermo Scientific). The HPLC conditions were previously
265 described by Corona *et al.,* (20) with some modifications. Briefly, reverse phase separations
266 were carried out using a 100 x 2.1 mm i.d. 1.8 µm Zorbax SB C18 (Agilent) maintained at 25
267 °C and eluted at a flow rate of 0.2 mL/min with a 50 min gradient of 3-70% of 0.1% acidic
268 methanol in 0.1% aqueous formic acid. After passing through the flow cell of the PDA detector
269 the column eluate was directed to an ExactiveTM Orbitrap mass spectrometer fitted with a
270 heated electrospray ionization probe (Thermo Scientific) operating in negative ionization
271 mode. Analyses were based on scanning from 100 to 1000 m/z, with in-source collision-
272 induced dissociation at 25.0 eV. The capillary temperature was 350 °C, the heater temperature
273 was 150 °C, the sheath gas and the auxillary gas flow rate were both 25 and 5 units,
274 respectively, and the sweep gas was 4 and the spray voltage was 3.00 kv. Data acquisition and
275 processing were carried out using Xcalibur 3.0 software.

276 Untargeted analysis of the selected urine and plasma samples was performed using mass
277 spectral data from the orbitrap analysis applied to the Compound Discoverer software (version
278 2, Thermo Fisher Scientific Inc.). The Compound Discover application processes the raw data

into workflows that can be defined on the basis of the nature of the experimental setup. In our case, the workflow selected was 'untargeted metabolomics workflow' that includes retention time alignment, component detection, grouping, elemental composition prediction, gap filling, hide chemical background (using blanks), ID using mzCloud and ChemSpider and differential analysis. The parameters were adjusted to our experimental conditions. Samples were grouped and labelled according to our experimental design, either before or after supplementation of seaweed capsule, the output, as peak areas for the detected peaks was used to develop a multivariate data analysis by Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA). Analysis of seaweed phenolics in blood and urine was also undertaken by HPLC-DAD analysis (**Supplemental Tables 1 & 2**) in a manner consistent with previous studies (15, 20).

Power calculations and statistical analyses

Power calculations were performed for the primary endpoint of the change in DNA damage in peripheral blood mononuclear cells. Based on data from a previous study (24), 72.6 participants were needed to detect a 25% change in DNA damage in lymphocytes (α 0.05).

All values are expressed as mean ± SD, unless otherwise specified. The mean values are reported for all participants (n=78) during both treatment phases (SPE, Maltodextrin). Significant associations between several outcomes and confounders including BMI and gender were identified at baseline using bivariate correlations or independent t-tests, as appropriate (TOC & Gender P=0.03, DNA damage & BMI P=0.048, DNA damage & Gender P=0.009). Consequently, data was also analysed by stratification of increasing risk, including overweight participants (n=42) and obese participants (n=36). The subgroup analysis was not preplanned; therefore, are not powered adequately and should be considered exploratory. All biochemical analysis was conducted in duplicates, unless otherwise stated, and the mean values taken as the final result. For all markers, the results are presented as treatment effects. This was undertaken

by calculating individual differences between pre– and post– values for both control and treatment phases for each subject. Paired T-tests were then carried out on the difference scores (post-treatment value *minus* pre-treatment values) for both treatment phases (SPE and Maltodextrin). Significance level was set at $P<0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 22.0 (SPSS Inc., Chicago, IL, USA).

Multivariate Data Analysis

Data were obtained as peak areas from the Compound Discover automatic integration software and consisted of 2194 and 3289 potential metabolites (or features) in urine and plasma samples, respectively. Relative peak areas of the metabolites (normalized by the total urine excretion of each subject) obtained by UPLC-HRMS were imported into MATLAB R2015b (Mathworks, USA). PLS toolbox v.8.5 (Eigenvector, USA) and homemade scripts were used. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the most widely used tools to explore similarities and patterns among samples where data grouping are unclear. Moreover, the orthogonal partial least squares discriminate analysis (OPLS-DA) method was performed as a typical supervised multivariate methodology used in metabolomics studies (30, 31). Several data pre-processing transformations were performed and evaluated, such as Probabilistic Quote Normalization (PQN), Log transformation, mean centering, pareto scaling and auto-scaling. In our case, PQN and autoscaling were selected as pre-processing techniques to reach the lowest root mean square error (RMSE) in an iterative process. A re-sampling method, cross-validation k-fold cross validation (k=5) was also used to evaluate the number of latent variables (according to the lowest RMSE) and the prediction ability of our models. To reduce the impact of the random split of CV-participants, the mean values of the estimated results were obtained after 20 random 5-fold CV. Urine models provided successful

classification results at the cross validation step and achieved good prediction parameters that can be explained by the area under the curve values (1.00 and 0.895). Based on the plasma data set and classes selected a discriminant model could not be developed. Moreover, the Variable Importance in Projection (VIP) is the widely known metric that is used to identify potential markers in metabolomics studies (32). VIP is a weighted sum of squares of the PLS weight which indicates the importance of the variable to the whole model. The cut off VIP value selected in our study was 2. The potential markers were extracted from the two models to compare the results obtained.

RESULTS

Baseline characteristics

Eighty participants (males n=39, females n=41) were enrolled on this 24-week randomised, double-blind, placebo-controlled crossover trial. The intervention was conducted as per the protocol and there were no adverse events associated with the intervention. The study population had a mean group age of 42.7±7.1 years and a mean BMI of 30.2±3.9 kg/m^2. The study had an overall compliance of 97% with 78 participants completing the 24-week study; two participants withdrew from the study at the midpoint for personal reasons and compliance was not significantly different by treatment group or time period (P>0.05) (**Figure 1**). There were no significant differences between the participants in age and physical characteristics at the beginning of either treatment phase (**Table 2**).

Habitual dietary intake

Dietary analysis of habitual intake (midpoint) during both treatment phases (SPE, Maltodextrin) of the crossover trial is described in **Table 3**. There were no significant differences between treatment phases for any of the macronutrients or micronutrients analysed

354 indicating that the seaweed phenolic extract did not affect the habitual food consumption
355 patterns in the study population.
356
357 **Effects of seaweed (poly)phenol extract on DNA damage**
358 Basal levels of DNA damage observed in the study were consistent with previous studies
359 with a mean group average of 6.72 ± 2.48% tail DNA (data not shown). In response to an
360 oxidative challenge with 150 µM H_2O_2, DNA damage was increased to an average of
361 approximately 34 ± 7% tail DNA in both the placebo and SPE phase in all participants (**Table**
362 **4)**. The 8-week intervention with a seaweed phenolic extract resulted in a significant reduction
363 in basal DNA damage, as measured by the Comet assay, in obese participants only (BMI >30
364 kg/m^2), with a significant reduction (P=0.044) in basal DNA damage observed (**Table 4**). A
365 significant reduction was not observed in the total population (n=78) nor in participants
366 classified as overweight (n=42), either in terms of a challenge response or in basal levels. In
367 addition, we noted that consumption of seaweed phenolic extract also significantly reduced
368 (P=0.009) basal DNA damage in males only with a mean group change value of -0.8 ± 2.5 %
369 tail DNA (SPE) compared to 0.9 ±2.8% tail DNA in the control. No significant effects were
370 observed for females.
371
372 **Total oxidative capacity of seaweed phenolics**
373 Total oxidative capacity (peroxide levels) in plasma samples from all participants (n=78),
374 in overweight participants (n=42) and in obese participants (n=36) were measured after the
375 placebo and seaweed capsule intervention (**Figure 2**). There were no significant changes from
376 baseline after either treatment phase (SPE, Maltodextrin) in all participants (n=78) nor in
377 overweight (n=42) or obese (n=36) sub groups. However consumption of seaweed phenolic
378 extract also significantly reduced (P=0.018) TOC in females only with a mean group change

379 value of -7.44 ± 29.37 µM peroxides (SPE) compared to 4.33 ± 22.36 µM peroxides in the
380 control. No significant effects were observed for males.
381
382 **Effects of seaweed (poly)phenol extract on blood lipids and CRP**
383 Analysis of Pre- and post- values, and the percentage change, for both the placebo phase
384 and the SPE phase for each blood lipid biomarker and CRP indicated that the 8-week
385 supplementation with seaweed (poly)phenol extract did not significantly affect any
386 cardiovascular risk marker **(Table 4).**
387
388 **Effects of seaweed (poly)phenol extract on inflammatory markers**
389 No significant differences were measured in any of the inflammatory cytokines in either
390 treatment phase (SPE, Maltodextrin) for all study participants (n=78) (**Figure 4**) and in addition
391 no significant effects on the cytokine profiles were observed.
392
393 **Bioavailability of seaweed phenolics, untargeted analysis**
394 Initially an unsupervised PCA model was carried out (**Figure 4A**) on the urine data from all
395 78 participants. The first component of the PC1 vs PC2 scores plot obtained from the data set
396 explained 23.14% of the total variance showing a clear trend among some of the participants
397 compared to the rest. Moreover, a hierarchical cluster analysis was performed to verify this
398 natural aggrupation pattern among the 78 participants suggesting, two distinct clusters among
399 all the participants (**Figure 4B**), accounting for the inter-individual differences associated with
400 variation in the urinary metabolite profiles. Recent studies suggest that individuals can be
401 clustered into distinct groups based on their gut microbiome composition, functional
402 metabolism (33) or individual responses to obtain markers of a specific treatment, minimizing
403 the inter-individual data that are not the target of the study. In order to elucidate the observed

variability between these two groups, two supervised models were performed, one with the urinary profiles from all the participants before the seaweed consumption and another one with the urinary profiles from all the participants after seaweed consumption. Both supervised models verified differences between the two groups of individuals (Group 1 and Group 2) and provided successful classification results at the cross validation step and achieved in the pre-treatment and post-treatment samples 95% and 98% sensitivity (participants of the class of interest correctly assigned to their class) and 100% and 100% specificity (participants not belonging to the class of interest were correctly not assigned to that class), respectively (**Supplemental Figure 3**). It is noteworthy that even stratifying for BMI category, the multivariate analysis of the urine profiles did not show clear differentiation of the metabolome between obese or overweight individuals.

By using the preliminary information provided by PCA and HCA related to the stratification of the individuals into groups which share a common excretion profile before and after supplementation with seaweed capsules, two data sets were analysed. The individuals were stratified into two groups; Group 1 including 70 participants; and Group 2 including 8 participants (S58, S60, S61, S71, S76, S78, S79, S83, all being overweight individuals). Two supervised OPLS-DA models were built to discriminate according to the seaweed treatment in both models (**Figure 5**). In our study we used two latent variables (LV) and the sensitivity and specificity values were set to 100% at the calibration step recognition ability of the two models. These analyses showed that the human urine metabolome in both groups of participants was modified after the 8-week supplementation with seaweed (poly)phenol extract compared to baseline urine metabolic profiles. Using the loadings plot and the variable importance in projection values (VIP) we ascertained important contributors between the modelled classes and therefore identified the compounds responsible for the difference in the urine metabolic profile before and after 8-weeks seaweed (poly)phenol extract consumption in both groups.

VIP is the widely known metric that is used to identify potential markers (metabolites) in metabolomics studies. Further, the urine scores plot allowed the identification of those metabolites which appeared after the seaweed (poly)phenol extract intake (**Figure 5**) in both groups of participants.

The main contributors to metabolome differentiation in the urine before and after seaweed consumption in both groups of participants are described in **Supplemental Tables 3 & 4**. Positive loading values (x axis) and higher VIP values (VIP>2.0) were detected in urine after seaweed consumption and were the responsible of the observed differences between the participants classes (post ingestion vs non ingestion of seaweed (poly)phenol extract). Urine metabolites tentatively identified as pyrogallol/phloroglucinol sulfate, hydroxytrifurahol A-glucuronide and dioxinodehydroeckol glucuronide are considered clear biomarkers of seaweed consumption in Group 1 while C-O-C dimer of phloroglucinol-sulfate, C-O-C-dimer of phloroglucinol, fucophloroethol-glucuronide, diphlorethol sulfates and dioxinodehydroeckol glucuronide are those biomarkers of seaweed consumption in Group 2. All of them have shown high values in the variable importance in the projection (values > 2) (**Table 5).**

Based upon the identified seaweed (poly)phenol metabolites, the total amount of seaweed metabolites excreted in urine varied noticeably between participant ranging from 0.001 to 4.140 m moles, with a group average of 1.29 +/- 0.88 m moles. Some 25% of the population (19/78) appeared to be low excretors, with urinary seaweed metabolites excretion less than 0.5 m moles, while 55% of the population (43/78) were medium excretors (0.5 and 2 m moles) and the remaining 20% of the population (16/78) were high excretors (>2 m moles) (**Supplemental Table 5**). Despite exploration using PCA or HCA, the plasma metabolome did not allow a clear differentiation between participants in either treatment phase (data not shown). As a result, it

453 was not possible to define exposure biomarkers in plasma samples across all the participants
454 (n=78).

455

456 **DISCUSSION**

457 The association of seaweed consumption with reduced CVD risk factor has been tested
458 largely within *in vitro* or animal models, with only limited human data allowing substantiation
459 of the proposed beneficial properties of seaweeds (12, 13, 34). The work reported in this paper
460 represents the first comprehensive study of the *in vivo* bioactivity and bioavailability of
461 seaweed (poly)phenolics on biomarkers of inflammation and oxidative stress in overweight
462 and obese individuals. Participant retention rates were high (98%) and self-reported SPE
463 capsule intake (97%) indicated that the intervention was implemented successfully in this
464 group of participants. There was, however, no significant difference between the intervention
465 (SPE capsule) and control (placebo capsule) phases for markers of oxidative stress, antioxidant
466 status or inflammation in the population as a whole (n=78). Stratifying for BMI however
467 revealed a significant albeit modest decrease in DNA damage in the obese population (n=36).
468 This is consistent with Park *et al*. (35) who tested the natural microalgal carotenoid astaxanthin,
469 in 14 healthy females (2 mg extract/d for 8 weeks) and showed a significant reduction in the
470 oxidative damage plasma marker 8-hydroxy-2'-deoxyguanosine. Obese individuals are
471 associated with increased basal DNA damage, higher oxidant status and increased oxidative
472 damage to macromolecules as a group and are thus at higher risk of chronic disease (36-39). In
473 the current work, an overweight/obese population (age 30-65 years, BMI >25 kg/m^2)
474 supplementation with seaweed (poly)phenolics significantly reduced basal levels of
475 lymphocyte DNA damage (by 23%) but only in the obese sub-population (n=36). These two
476 seaweed-extract interventions are consistent with data from *in vitro* studies which demonstrated
477 anti-genotoxic activity for seaweed extracts on a range of cell lines (40,41), including the SPE

478 extract used in our study (15). The biological mechanism underpinning the observed reduction
479 in DNA damage in the obese group could not be determined from our data, however the
480 activation of the cytoprotective Nrf2/ARE pathway may be involved, as seaweed (poly)phenols
481 such as eckol are anti-genotoxic (42) and can activate Nrf2-mediated HO-1 induction (43,44)
482 consistent with effects observed for (poly)phenols from terrestrial sources (45,46). We
483 observed a 28% decrease in CRP levels, as a marker of inflammation, in response to seaweed
484 extract consumption however this change was not significant, in contrast to the observations
485 of Park *et al*. (35). However, a study investigating the effect of consuming *Palmaria palmata*
486 (5 g/day) incorporated into bread found that it significantly increased CRP by 16%, suggesting
487 that *P. palmata* stimulates inflammation rather than reducing it (47). Park *et al*. (35) reported
488 that there was no difference in TNF and IL-2 concentrations, but plasma IFN-γand IL-6
489 increased on week 8 in participants given 8mg astaxanthin. Within our study, we similarly
490 observed no significant changes in IL-2 or TNF, nor did we see an alteration in IFN-γor IL-6.
491 The seaweed extract tested did not affect immune function (cell mediated and humoral immune
492 responses as tested by the cytokine markers) following supplementation.rather than reducing it
493 (47). Park *et al.* (35) reported that there was no difference in TNF and IL-2 concentrations, but
494 plasma IFN-γand IL-6 increased on week 8 in participants given 8mg astaxanthin. Within this
495 study, we similarly observed no significant changes in IL-2 or TNF, nor did we see an alteration
496 in IFN-γor IL-6. The seaweed extract tested did not affect immune function (cell mediated and
497 humoral immune responses as tested by the cytokine markers) following supplementation.
498
499 A comparison of metabolic profiles between urine and plasma samples of 78 individuals who
500 consumed seaweed capsules has allowed evaluation of seaweed molecules potentially
501 responsible for the modest beneficial effects observed *in vivo* as well as identifying biological
502 markers linked to seaweed consumption. We determined differences in the urine metabolite

503 profiles between participants, and thus were able to stratify the individuals into groups that
504 shared common excretion metabolite profiles both pre- and post- seaweed supplementation.
505 Despite substantial inter-individual variation in the concentration of seaweed metabolites
506 excreted in urine (0.001 to 4.140 m moles, the urinary profiles of these two groups were
507 statistically different and we have been able to select the metabolites responsible for this
508 discrimination as potential biomarkers of seaweed consumption (**Table 5**). Our comprehensive
509 multivariate analysis of the metabolite profiles showed the expected person-to-person variation
510 in the 0-24h urinary excretion of seaweed (poly)phenols, which could be attributed to
511 differences in gut microbiota or living conditions amongst study participants. Nonetheless, we
512 could group the 78 participants by ability to metabolize seaweed (poly)phenols. Group 1
513 individuals were characterized by greater excretion of seaweed–derived metabolites such as
514 pyrogallol/phloroglucinol sulfate, hydroxytrifurahol A-sulfate and dioxinodehydroeckol, while
515 Group 2 individuals were characterized by excretion of C-O-C dimer of phloroglucinol-sulfate,
516 C-O-C dimer of phloroglucinol, fucophloroethol-glucuronide, diphlorethol sulfates and
517 dioxinodehydroeckol glucuronide. This inter-individual variation in absorption of seaweed
518 polyphenols is consistent with profiles previously observed in the bioavailability of dietary
519 (poly)phenols such as orange, cranberry and pomegranate juices (48-50).

520

521 The seaweed phenolic metabolites and can be divided into (a) phase II sulphated and
522 glucuronidated metabolites related to the targeted components described earlier by Corona *et*
523 *al.* (20) (**Supplemental Tables 1 & 2**); arguably formed in the liver, and (b) an extended list
524 of unknown compounds which could be potential breakdown products or metabolites of the
525 original seaweed (poly)phenols catabolised by colonic bacteria. However, further investigation
526 would be required to confirm the identity and origin of these unknown compounds. We
527 previously reported the poor absorption of the high molecular weight phlorotannins in the upper

gastrointestinal tract (20) likely results in them reaching the colon and becoming subject to microbial fermentation to lower molecular weight derivatives, as we have recently shown using an in vitro gut microbiota model (15). The urinary metabolite profiles of seaweed phenolics from the low and high excretors clearly indicated a high inter-individual variation in metabolism. It is possible that inter-individual variation in gut microbiota underlie these metabolic changes and were responsible for the observed differences (51-53). The study of seaweed polyphenol(s) bioavailability remains challenging due to the high range of molecular weight compounds present, and their characterisation is complicated further by the lack of commercially available standards. Other limitations of the present study include a relatively short-term intervention, and thus results cannot be extrapolated to long-term chronic consumption. In addition, study participants were mostly recruited from the University of Ulster staff and local residents and thus may not be representative of the general overweight population of Northern Ireland. While lymphocyte DNA damage (spontaneous DNA SBs) is considered appropriate for the substantiation of EFSA health claims in the context of protection against generic DNA damage, a lesion specific enzyme such as (ENDO III) would have been required for a claim related to oxidative DNA damage (54). With respect to the TOC assay it is based on the reaction of endogenous peroxides with hydrogen peroxide, using TMB as the chromogenic substrate, and provides a measure of total peroxide levels in plasma. While this assay lacks specificity in comparison to other measures of oxidative stress, the direct correlation between oxygen radicals and peroxides allows measurement and characterization of the oxidative status/oxidative stress in biological fluids. Moreover the test is characterized by linearity, good precision, and endpoint determination (55). Finally, the study population number may have been too small to yield significant results in intercellular cytokines. These factors should be considered for the design of future studies in this area.

552 In conclusion, this work represents the first comprehensive human study investigating
553 bioavailability and metabolism of seaweed (poly)phenolics. Consumption of SPE decreased
554 DNA damage–albeit to a modest extent–in obese individuals only, with no clear effects on
555 clinical markers of inflammation. Untargeted analysis identified novel urinary biomarkers of
556 seaweed consumption and highlighted a high degree of inter-person variation in the metabolism
557 of seaweed phenolics. Future studies that address the ingestion of seaweed phenolics will need
558 to consider and adjust for these parameters, and this work has highlighted the importance of
559 establishing an individual's capacity for metabolising (poly)phenols.
560

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575

References

1. Tresserra-Rimbau A, Rimm EB, Medina-Remón A, Martínez-González MA, de la Torre R, Corella D, Salas-Salvadó J, Gómez-Gracia E, Lapetra J, Arós F et al; PREDIMED Study Investigators. Inverse association between habitual (poly)phenol intake and incidence of cardiovascular events in the PREDIMED study. Nutr Metab Cardiovasc Dis 2014;24(6):639-647.
2. Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, Tjønneland A, Kyrø C, Fagherazzi G, Boutron-Ruault MC, et al. Dietary (poly)phenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Nutr 2016;55(4):1359-75.
3. Rienks J, Barbaresko J, Nöthlings U. Association of (poly)phenol biomarkers with cardiovascular disease and mortality risk: a systematic review and meta-analysis of observational studies. Nutrients 2017; 9(4): 415-426.
4. Cardoso SM, Pereira O, Seca AM, Pinto DC, Silva AM. Seaweeds as Preventive Agents for Cardiovascular Diseases: From Nutrients to Functional Foods. Mar Drugs. 2015 Nov 12;13(11):6838-65.
5. Connan S, Deslandes E, Gall EA. Influence of day–night and tidal cycles on phenol content and antioxidant capacity in three temperate intertidal brown seaweeds. J Exp Mar Biol Ecol 2007;349(2):359-369.
6. Cruces E, Huovinen P, Gomez I. Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three south Pacific kelps. Photochem Photobiol 2012;88(1):58-66.
7. Koivikko R, Eranen JK, Loponen J, Jormalainen V. Variation of phlorotannins among three populations of *Fucus vesiculosus* as revealed by HPLC and colorimetric quantification. J Chem Ecol 2008;34(1):57-64.

8. Rioux LE, Turgeon SL, Beaulieu M. Effect of season on the composition of bioactive polysaccharides from the brown seaweed *Saccharina longicruris*. Phytochemistry 2009;70(8):1069-1075.
9. Cornish ML, Garbary DJ. Antioxidants from macroalgae: potential applications in human health and nutrition. Algae 2010;25(4):155-171.
10. Wijesekara I, Yoon NY, Kim S-K. Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits. Biofactors 2010;36(6): 408–414.
11. Hotchkiss S, Murphy C. (2014) Marine Macroalgae and Human Health. In: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology, edited by Pereira L & Neto J-M. CRC Press 2014. Pages 320–356
12. Brown EM, Allsopp PJ, Magee PJ, Gill CIR, Nitecki S, Strain CR, McSorley EM. Seaweed and human health. Nutr Rev 2014;72(3):205-216.
13. Brownlee I, Fairclough A, Hall A, Paxman J. 2012. The potential health benefits of seaweed and seaweed extract. pp. 119-136. In: V.H. Pomin, Vitor H., (ed.). Seaweed: ecology, nutrient composition and medicinal uses. Marine Biology: Earth Sciences in the 21st Century. Hauppauge, New York, Nova Science Publishers.
14. Kim M-M, Kim S-K. Effect of phloroglucinol on oxidative stress and inflammation. Food and Chemical Toxicology 2010;48(10):2925–2933.
15. Corona G, Coman MM, Guo Y, Hotchkiss S, Gill C, Yaqoob P, Spencer JPE, Rowland I. Effect of simulated gastrointestinal digestion and fermentation on polyphenolic content and bioactivity of brown seaweed phlorotannin-rich extracts. Mol Nutr Food Res. 2017 Nov;61(11). doi: 10.1002/mnfr.201700223. Epub 2017 Aug 29.
16. Zubia M, Fabre MS, Kerjean V, Le Lann K, Stiger-Pouvreau V, Fauchon M, Deslandes E. Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. Food Chem 2009;116:693–701.

17. O'Sullivan AM, O'Callaghan YC, O'Grady MN, Queguineur B, Hanniffy D, Troy DJ, Kerry JP, O'Brien NM. *In vitro* and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. Food Chem 2011;126(3):1064–1070.
18. Rajauria G, Jaiswal AK, Abu-Gannam N, Gupta S. Antimicrobial, Antioxidant and Free Radical-Scavenging Capacity of Brown Seaweed *Himanthalia Elongata* from Western Coast of Ireland. J Food Biochem 2013;37:322-335.
19. Keyrouz R, Abasq ML, Le Bourvellec C, Blanc N, Audibert L, ArGall E, Hauchard D. Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. Food Chem 2011;126:831–836.
20. Corona G, Ji Y, Anegboonlap P, Hotchkiss S, Gill CIR, Yaqoob P, Spencer JP, Rowland I. Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. Br J Nutr 2016;14;115(7):1240-53.
21. Paradis M-E, Couture P, Lamarche B. A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (*Ascophyllum nodosum* and *Fucusvesiculosus*) on post-challenge plasma glucose and insulin levels in men and women. Applied Physiology, Nutrition, and Metabolism 2011;36(6):913-919.
22. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer J Enol Viticult 1965;16: 144-58.
23. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low-levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-191.
24. Gill CIR, Haldar S, Boyd A, Bennett R, Whitefield J, Butler M, Pearson J, Bradbury I, Rowland I. Watercress supplementation in diet reduces lymphocyte DNA damage

and alters blood antioxidant status in healthy participants. Am J Clin Nutr 2007;85:504-510.

25. Martini D, Rossi S, Biasini B, Zavaroni I, Bedogni G, Musci M, Pruneti C, Passeri G, Ventura M, Di Nuzzo S, et al. Claimed effects, outcome variables and methods of measurement for health claims proposed under European Community Regulation 1924/2006 in the framework of protection against oxidative damage and cardiovascular health. Nutr Metab Cardiovasc Dis. 2017 Jun;27(6):473-503.
26. Josephy PD, Eling T, Mason RP. The horseradish peroxidase-catalyzed oxidation of 3,5,3',5'-tetramethylbenzidine. Free radical and charge-transfer complex intermediates. J Biol Chem 1982;257(7):3669-75.
27. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
28. Pereira-Caro G, Ludwig IA, Polyviou T, Malkova D, García A, Moreno-Rojas JM, Crozier A. Identification of plasma and urinary metabolites and catabolites derived from orange juice (poly)phenols: analysis by high-performance liquid chromatography-high-resolution mass spectrometry. J. Agric. Food Chem. 2016: 64: 5724-5735.
29. Laird E, McNulty H, Ward M, Hoey L, McSorley E, Wallace JMW, Carson E, Molloy AM, Healy M, Casey C et al. Vitamin D deficiency is associated with inflammation in older Irish adults. J Clin Endocrinol Metab 2014;99(5):1807-15.
30. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: state of the art in 2015. Frontiers in bioengineering and biotechnology 2015;3:23.
31. Gromski PS, Muhamadali H, Ellis DI, Xu Y, Correa E, Turner ML, Goodacre R. A tutorial review: Metabolomics and partial least squares-discriminant analysis–a

marriage of convenience or a shotgun wedding. Analytica chimica acta 2015;879:10-23.

32. Yi L, Dong N, Yun Y, Deng B, Ren D, Liu S, Liang Y. Chemometric methods in data processing of mass spectrometry-based metabolomics: A review. Analytica chimica acta 2016;914:17-34.
33. Lampe JW, Navarro SL, Hullar MA, Shojaie A.Inter-individual differences in response to dietary intervention: integrating omics platforms towards personalised dietary recommendations. Proc Nutr Soc. 2013;72(2):207-18.
34. Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI *et al*. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology 2007;17(5):541-552.
35. Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab (Lond). 2010;7:18.
36. Bukhari SA, Rajoka MI, Nagra SA, Rehman ZU. Plasma homocysteine and DNA damage profiles in normal and obese participants in the Pakistani population. Mol Biol Rep 2010; 37: 289–295.
37. Karaman A, Aydın H, Geçkinli B, Çetinkaya A, Karaman S. DNA damage is increased in lymphocytes of patients with metabolic syndrome. Mutat Res Genet Toxicol Environ Mutagen. 2015;782:30-35
38. Karbownik-Lewinska M, Szosland J, Kokoszko-Bilska A, Stępniak J, Zasada K, Gesing A, Lewinski A. Direct contribution of obesity to oxidative damage to macromolecules. Neuro Endocrinol Lett. 2012;33(4):453-61.

39. Monzo-Beltran L, Vazquez-Tarragón A, Cerdà C, Garcia-Perez P, Iradi A, Sánchez C6, Climent B, Tormos C, Vázquez-Prado A, Girbés J, Estáñ N, Blesa S, Cortés R, Chaves FJ, Sáez GT.One-year follow-up of clinical, metabolic and oxidative stress profile of morbid obese patients after laparoscopic sleeve gastrectomy. 8-oxo-dG as a clinical marker. Redox Biol. 2017;12:389-402

40. O'Sullivan AM, O'Callaghan YC, O'Grady MN, Queguineur B, Hanniffy D, Troy DJ, Kerry JP, O'Brien NM. Assessment of the ability of seaweed extracts to protect against hydrogen peroxide and tert-butyl hydroperoxide induced cellular damage in Caco-2 cells. Food Chem 2012;134(2):1137-40.

41. Yang J-I, Yeh C-C, Lee J-C, Yi S-C, Huang H-W, Tseng C-N, Chang H-W. Aqueous Extracts of the Edible *Gracilaria tenustipitata* are Protective Against H_2O_2-Induced DNA Damage, Growth Inhibition, and Cell Cycle Arrest. Molecules 2012;17:7241-7254.

42. Piao MJ, Lee NH, Chae S, Hyun JW. Eckol inhibits ultraviolet B-induced cell damage in human keratinocytes via a decrease in oxidative stress. Biol Pharm Bull. 2012;35(6):873-80.

43. Kim KC, Kang KA, Zhang R, Piao MJ, Kim GY, Kang MY, Lee SJ, Lee NH, Surh YJ, Hyun JW. Up-regulation of Nrf2-mediated heme oxygenase-1 expression by eckol, a phlorotannin compound, through activation of Erk and PI3K/Akt. Int J Biochem Cell Biol. 2010;42(2):297-305.

44. Yang YI, Woo JH, Seo YJ, Lee KT, Lim Y, Choi JH. Protective Effect of Brown Alga Phlorotannins against Hyper-inflammatory Responses in Lipopolysaccharide-Induced Sepsis Models. J Agric Food Chem. 2016 Jan 27;64(3):570-8.

45. Yang YC, Lii CK, Lin AH, Yeh YW, Yao HT, Li CC, Liu KL, Chen HW. Induction of glutathione synthesis and heme oxygenase 1 by the flavonoids butein and phloretin is

mediated through the ERK/Nrf2 pathway and protects against oxidative stress. Free Radic Biol Med. 2011 Dec 1;51(11):2073-81.

46. McDougall GJ, Allwood JW, Pereira-Caro G, Brown EM, Verrall S, Stewart D, Latimer C, McMullan G, Lawther R, O'Connor G, Rowland I, Crozier A, Gill CI. Novel colon-available triterpenoids identified in raspberry fruits exhibit antigenotoxic activities in vitro. Mol Nutr Food Res. 2017 Feb;61(2).
47. Allsopp P, Crowe W, Bahar B, Harnedy PA, Brown ES, Taylor SS, Smyth TJ, Soler-Vila A, Magee PJ, Gill CIR, et al . The effect of consuming *Palmaria palmata* enriched bread on inflammatory markers, antioxidant status, lipid profile and thyroid function in a randomised placebo controlled intervention trial in healthy adults. Eur J Nutr 2016;55(5):1951-62.
48. Pereira-Caro G, Polyviou T, Ludwig IA, Nastase AM, Moreno-Rojas JM, Garcia AL, Malkova D, Crozier A. Bioavailability of orange juice (poly)phenols: the impact of short-term cessation of training by male endurance athletes. Am J Clin Nutr. 2017 Sep;106(3):791-800.
49. Feliciano RP, Mills CE, Istas G, Heiss C, Rodriguez-Mateos A. Absorption, Metabolism and Excretion of Cranberry (Poly)phenols in Humans: A Dose Response Study and Assessment of Inter-Individual Variability. Nutrients. 2017 Mar 11;9(3). pii: E268. doi: 10.3390/nu9030268.
50. González-Sarrías A, García-Villalba R, Romo-Vaquero M, Alasalvar C, Örem A, Zafrilla P, Tomás-Barberán FA, Selma MV, Espín JC. Clustering according to urolithin metabotype explains the interindividual variability in the improvement of cardiovascular risk biomarkers in overweight-obese individuals consuming pomegranate: A randomized clinical trial. Mol Nutr Food Res. 2017 May;61(5). doi: 10.1002/mnfr.201600830.

51. Tomás-Barberán FA., Selma MV, Espín JC. Interactions of gut microbiota with dietary (poly)phenol s and consequences to human health. Curr Opin Clin Nutr Metab Care 2016;19(6):471-476.
52. Manach C, Milenkovic D, Van de Wiele T, Rodriguez-Mateos A, de Roos B, Garcia-Conesa MT, Landberg R, Gibney ER, Heinonen M, Tomás-Barberán F, *et al*. Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy ageing and cardiometabolic risk reduction. Mol Nutr Food Res 2017;61(6).
53. van Duynhoven J, Vaughan EE, Jacobs DM, Kemperman RA, van Velzen EJ, Gross G, Roger LC, Possemiers S, Smilde AK, Doré J, et al. Metabolic fate of (poly)phenols in the human superorganism. Proc Natl Acad Sci USA 2011;108(Suppl1):4531-8.
54. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies),Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI,Mangelsdorf I, McArdle HJ, et al 2018. Guidance for the scientific requirements for health claims related to antioxidants,oxidative damage and cardiovascular health (Revision 1). EFSA Journal 2018;16(1):5136, 21 pp.https://doi.org/10.2903/j.efsa.2018.5136.
55. Tatzber F, Griebenow S, Wonisch W, Winkler R. Dual method for the determination of peroxidase activity and total peroxides-iodide leads to a significant increase of peroxidase activity in human sera. Anal Biochem. 2003;316(2):147-53.

Table 1. Key components of phlorotannin rich basic seaweed extract, High Molecular Weight (HMW) seaweed extract fraction, and blend used for intervention seaweed polyphenolic extract capsules (SPE).

Extract component	**Basic seaweed extract** mg/175mg of extract	**HMW seaweed extract fraction** mg/50mg of extract	**Blend (SPE capsule)** mg/400mg capsule
Phlorotannin	61.25	46.05	107.3
Iodine	0.48	0.02	0.5
Maltodextrin[1]	0	0	175
Minerals	39.38	13	52.38
Fucoxanthin	0	36.5	36.5
Laminarin as glucose	10.68	1.75	12.43
Fucoidan as fucose	0	<0.001	<0.001
Mannitol	29.23	5.9	35.13
Inorganic arsenic	<0.001	<0.001	<0.001
Cadmium (LD 0.15mg/kg)	<LD	<LD	<LD
Mercury (LD 0.016mg/kg)	<LD	<LD	<LD
Lead (LD 1.1mg/kg)	<LD	<LD	<0.001
Tin (LD 1.7mg/kg)	<LD	<LD	<LD

[1]maltodextrin was added to the capsule formulation as an excipient.

Table 2. Baseline characteristics of the study population (n=80).

Variable	**Placebo treatment phase (n=80)**	**SPE treatment phase (n=80)**
Age (y)	42.8 ± 7.2	42.9 ± 7.1
Gender (M/W)	20/20	19/21
Height (m)	1.71 ± 0.08	1.72 ± 0.10
Weight (kg)	88.9 ± 14.1	89.1 ± 17.3
BMI (kg/m^2)	30.3 ± 3.5	30.0 ± 4.4

No significant differences P>0.05, Paired T Test.

Table 3. Habitual nutrient intake of subjects during intervention study

Variable intake	**Placebo treatment phase (n=77)[1]**	**SPE treatment phase (n=77)**	**% Change[2]**	**P value[3]**
Energy (kcal/d)	1949 ± 590[4]	2057 ± 684	5	0.110
Protein (g/d)	79.4 ± 28.6	79.9 ± 25.3	0.5	0.782
Carbohydrate (g/d)	220.6 ± 72.1	231.3 ± 84.2	5	0.278
Total fat (g/d)	78.6 ± 26.5	85.2 ± 34.8	8	0.058
Saturated fat (g/d)	29.0 ± 10.2	30.7 ± 15.3	6	0.417
Monounsaturated fat (g/d)	24.4 ± 9.3	26.8 ± 12.4	9	0.112
Polyunsaturated fat (g/d)	12.1 ± 5.4	13.5 ± 6.4	10	0.059
Fibre (g/d)[5]	12.9 ± 6.2	13.3 ± 4.8	3	0.259
Vitamin C (mg/d)	71.8 ± 50.5	69.4 ± 47.1	-3	0.909
Vitamin E (mg/d)	7.17 ± 3.7	7.67 ± 4.0	7	0.279
Folate (µg/d)	223.9 ± 98.3	228.2 ± 85.5	2	0.370
Carotene (µg/d)	2821 ± 1900	2553 ± 1706	-11	0.185

[1]S66 did not complete a food diary in either phase. [2] Calculated from SPE phase – Placebo phase. [3] Mean treatment group values were not significantly different between phases, $P<0.05$ (Wilcoxin signed rank test). [4] Mean ± SD. [5]Calculated using the Englyst method.

Table 4. Effects of seaweed polyphenol extract on lymphocyte DNA damage, CRP and blood lipids.

	Total (n=78)				**Overweight (n=42)**				**Obese (n=36)**			
Blood marker	**Average baseline value**	**Placebo treatment effect**	**SPE treatment effect**	**P value**	**Average baseline value**	**Placebo treatment effect**	**SPE treatment effect**	**P value**	**Average baseline value**	**Placebo treatment effect**	**SPE treatment effect**	**P value**
DNA damage – basal (% Tail)	6.72 ± 2.48	0.74 ± 2.86	-0.41 ± 3.13	0.350	6.59 ± 2.80	0.32 ± 2.14	0.57 ± 3.24	0.129	6.91 ± 2.00	1.81 ± 4.50	0.15 ± 2.93	**0.044**
DNA damage - H_2O_2 (%Tail)	34.2 ± 7.00	-1.56 ± 6.60	-2.03 ± 6.40	0.390	35.2 ± 7.11	0.13 ± 2.19	0.76 ± 1.66	0.062	32.8 ± 6.69	1.26 ± 2.09	0.54 ± 1.54	0.111
CRP (mg/ml)	2.67 ± 3.9	0.01 ± 3.3	-0.83 ± 4.9	0.429	2.59 ± 4.69	0.00 ± 3.40	-1.32 ± 6.22	0.348	2.80 ± 2.56	-1.81 ± 11.82	0.72 ± 5.15	0.258
Cholesterol (mmol/l)	5.20 ± 0.77	-0.06 ± 0.57	-0.10 ± 0.57	0.256	5.18 ± 0.82	0.01 ± 0.55	-0.53 ± 0.54	0.201	5.24 ± 0.73	-0.13 ± 0.58	-0.15 ± 0.60	0.419
Triglycerides (mmol/l)	1.51 ± 0.94	0.01 ± 0.82	0.04 ± 0.96	0.385	1.34 ± 0.72	0.07 ± 0.79	-0.04 ± 0.48	0.278	1.75 ± 1.15	-0.06 ± 0.85	-0.02 ± 1.34	0.497
HDL (mmol/l)	1.37 ± 0.32	-0.01 ± 0.15	0.03 ± 0.15	0.187	1.46 ± 0.31	0.01 ± 0.16	-0.03 ± 0.15	0.150	1.25 ± 0.32	-0.04 ± 0.11	-0.04 ± 0.14	0.446
LDL (mmol/l)	3.16 ± 0.1	-0.08 ± 0.5	-0.06 ± 0.50	0.478	3.13 ± 0.69	-0.07 ± 0.57	0.01 ± 0.45	0.383	3.20 ± 0.67	-0.09 ± 0.56	-0.13 ± 0.56	0.412

Data is presented as treatment affects, calculated based on individual differences between pre- and post- values for both control and treatment phases for each subject. Paired T-tests were then carried out on the difference scores (post-treatment value *minus* pre-treatment values) between treatment (Seaweed phenolic extract capsule) and placebo control phase (maltodextrin). Significance level was set at $P<0.05$ (one-tailed T test).

Table 5. Phlorotannins metabolites tentatively identified in human urine samples from Group 1 (70 subjects) and Group 2 (8 subjects) after seaweed capsule consumption.

ID	VIP value	Rt (min)	Experimental Molecular Weight	Predicted Formula	Metabolite putative identification	Ratio[1]
Group 1 (72 subjects)						
652	2.06	25.6	205.9881	C6H6O6S	Pyrogallol/phloroglucinol sulfate	1.5
800	2.28	25.8	205.9881	C6H6O6S	Pyrogallol/phloroglucinol sulfate	1.2
1472	3.86	24.9	205.9881	C6H6O6S	Pyrogallol/phloroglucinol sulfate	1.1
1352	4.26	25.6	486.1727	C22H30O12	Hydroxytrifuhaol A-glucuronide	2.0
1453	3.83	25.4	486.1727	C22H30O12	Hydroxytrifuhaol A-glucuronide	1.9
1458	4.23	25.3	486.1727	C22H30O12	Hydroxytrifuhaol A-glucuronide	2.2
1483	4.39	25.3	486.1727	C22H30O12	Hydroxytrifuhaol A-glucuronide	2.2
1917	2.11	31.4	544.2881	C27H44O11	Dioxinodehydroeckol glucuronide	1.1
Group 2 (8 subjects)						
293	2.08	9.5	327.0951	C12H8O9S	C-O-C dimer of phloroglucinol-sulfate	1.2
702	2.20	24.1	248.0315	C12H8O6	C-O-C dimer of phloroglucinol	1.2
853	2.08	27.8	797.3186	C45H49O13	Fucophloroethol glucuronide	1.1
1293	2.28	34.2	330.1675	C12H10O9S	Diphlorethol sulfate	1.9
1356	2.31	34.1	330.1675	C12H10O9S	Diphlorethol sulfate	1.7
1633	2.07	35.1	544.2152	C27H44O11	Dioxinodehydroeckol glucuronide	1.2

ID, identification number. VIP (variable influence in projection) is a variable that summarizes the importance of X variables to the OPLS-DA model. Variables with values > 2 were the most influential in the model. All predicted formula derived with < 5 ppm mass accuracy data.

[1]Ratio: seaweed capsule consumption/placebo consumption.

FIGURE 1. CONSORT DIAGRAM. Progress of participants through the intervention study.

FIGURE 2. Comparison of the effects of SPE supplementation phase (□) with that of a placebo phase (•) on total oxidative capacity (TOC) in the total study population (n=78), in overweight (n=42), and in obese (n=36). All values were measured in duplicate expressed as mean ± SD. TOC is represented as the mean of the individual difference values (after – before supplementation) in the SPE and placebo treatment phases.

FIGURE 3. Comparison of the effects of SPE supplementation phase (■) with that of a placebo phase (□) on cytokine levels in the total study population (n=78), in overweight (n=42), and in obese (n=36). All values were measured in duplicate expressed as mean ± SD. Cytokines were measured as the mean of the individual difference value (after – before supplementation) in the SPE and placebo treatment phases. Change in mean treatment group values were not significantly different between treatment phases, $P>0.05$ (Paired T Test; one-tailed test). M.F.I.; Mean Fluorescence Intensity.

FIGURE 4. Principal Component Analysis (PCA) (A) and Hierachical Cluster Analysis Cluster (HCA) (B) of urinary profiles before (♦) and after (■) seaweed consumption by 80 participants. The HCA was calculated based on Eucledian distances and the Ward hierachical agglomerative method. The PC explained 23.14% of the total variance (PC-1 14.8% and PC-2 8.34 %).

FIGURE 5 A) OPLS-DA scores and B) loadings of the urine samples belong to group of participants 1 (70 participants). C) OPLS-DA scores and D) loading of urine samples belong to group of participants 2 (8 participants) before (♦) and after (■) seaweed ingestion. (Circles shown in the graph represent a confidence of 95%). LV1: latent variable 1; LV 2: latent variable 2. The cut off VIP value selected to be 2. For VIP scores identification see **Table 5** and Supplemental **Table 1**.

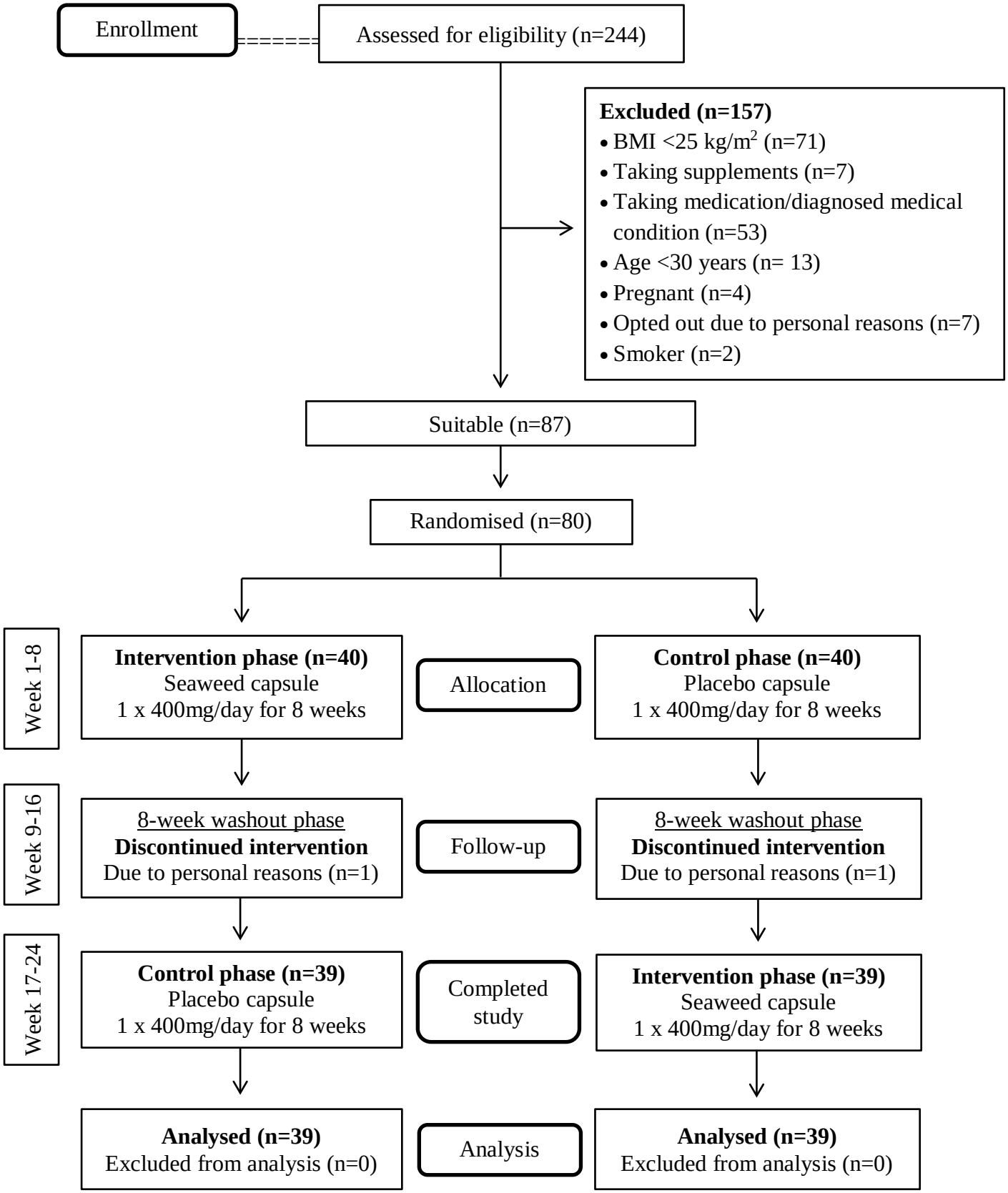

Enrollment
Assessed for eligibility (n=244)
Excluded (n=157)
• BMI <25 kg/m2 (n=71)
• Taking supplements (n=7)
• Taking medication/diagnosed medical condition (n=53)
• Age <30 years (n= 13)
• Pregnant (n=4)
• Opted out due to personal reasons (n=7)
• Smoker (n=2)
Suitable (n=87)
Randomised (n=80)
Week 1-8
Intervention phase (n=40)
Seaweed capsule
1 x 400mg/day for 8 weeks
Allocation
Control phase (n=40)
Placebo capsule
1 x 400mg/day for 8 weeks
Week 9-16
8-week washout phase
Discontinued intervention
Due to personal reasons (n=1)
Follow-up
8-week washout phase
Discontinued intervention
Due to personal reasons (n=1)
Week 17-24
Control phase (n=39)
Placebo capsule
1 x 400mg/day for 8 weeks
Completed study
Intervention phase (n=39)
Seaweed capsule
1 x 400mg/day for 8 weeks
Analysed (n=39)
Excluded from analysis (n=0)
Analysis
Analysed (n=39)
Excluded from analysis (n=0)

FIGURE 1.

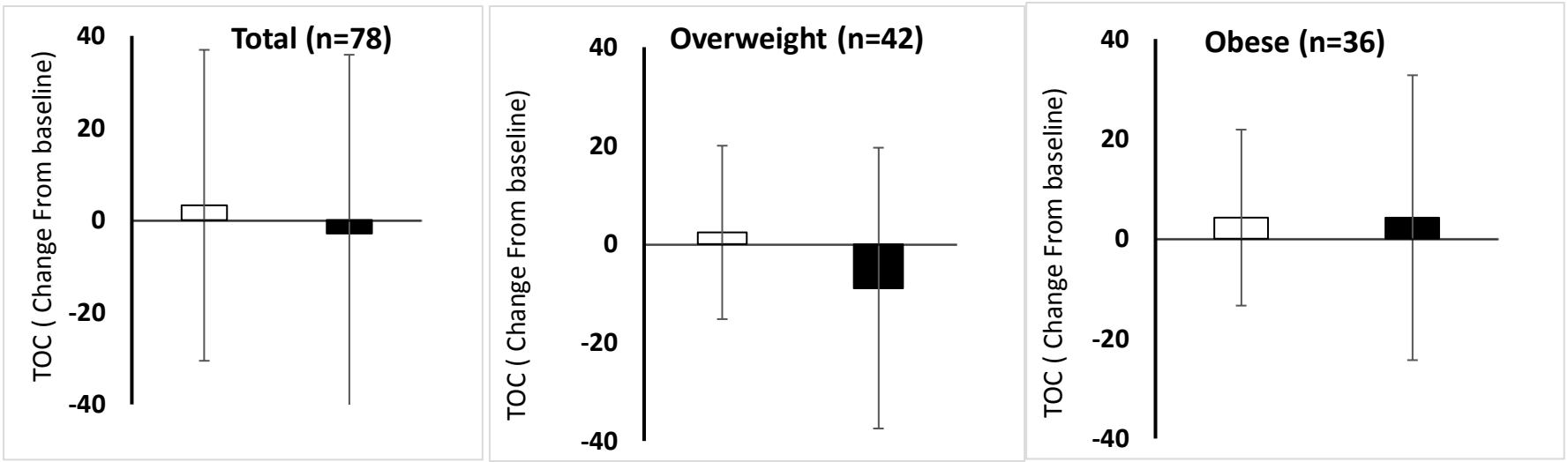

Total (n=78)
Overweight (n=42)
Obese (n=36)
TOC (Change From baseline)
40
20
0
-20
-40

FIGURE 2.

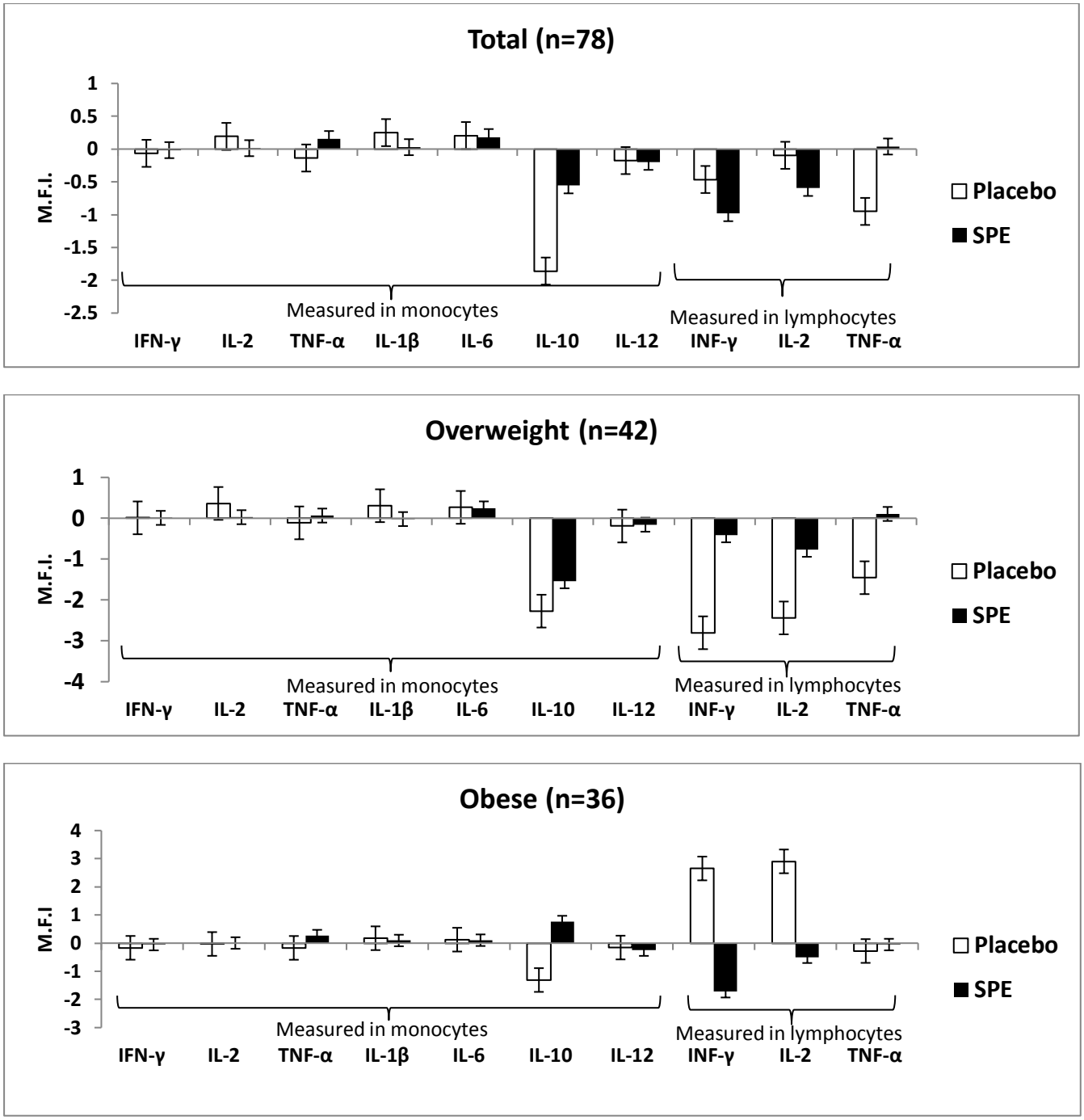

Total (n=78)
M.F.I.
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
Placebo
SPE
Measured in monocytes
Measured in lymphocytes
IFN-γ
IL-2
TNF-α
IL-1β
IL-6
IL-10
IL-12
INF-γ
IL-2
TNF-α
Overweight (n=42)
M.F.I.
1
0
-1
-2
-3
-4
Placebo
SPE
Measured in monocytes
Measured in lymphocytes
IFN-γ
IL-2
TNF-α
IL-1β
IL-6
IL-10
IL-12
INF-γ
IL-2
TNF-α
Obese (n=36)
M.F.I
4
3
2
1
0
-1
-2
-3
Placebo
SPE
Measured in monocytes
Measured in lymphocytes
IFN-γ
IL-2
TNF-α
IL-1β
IL-6
IL-10
IL-12
INF-γ
IL-2
TNF-α

FIGURE 3.

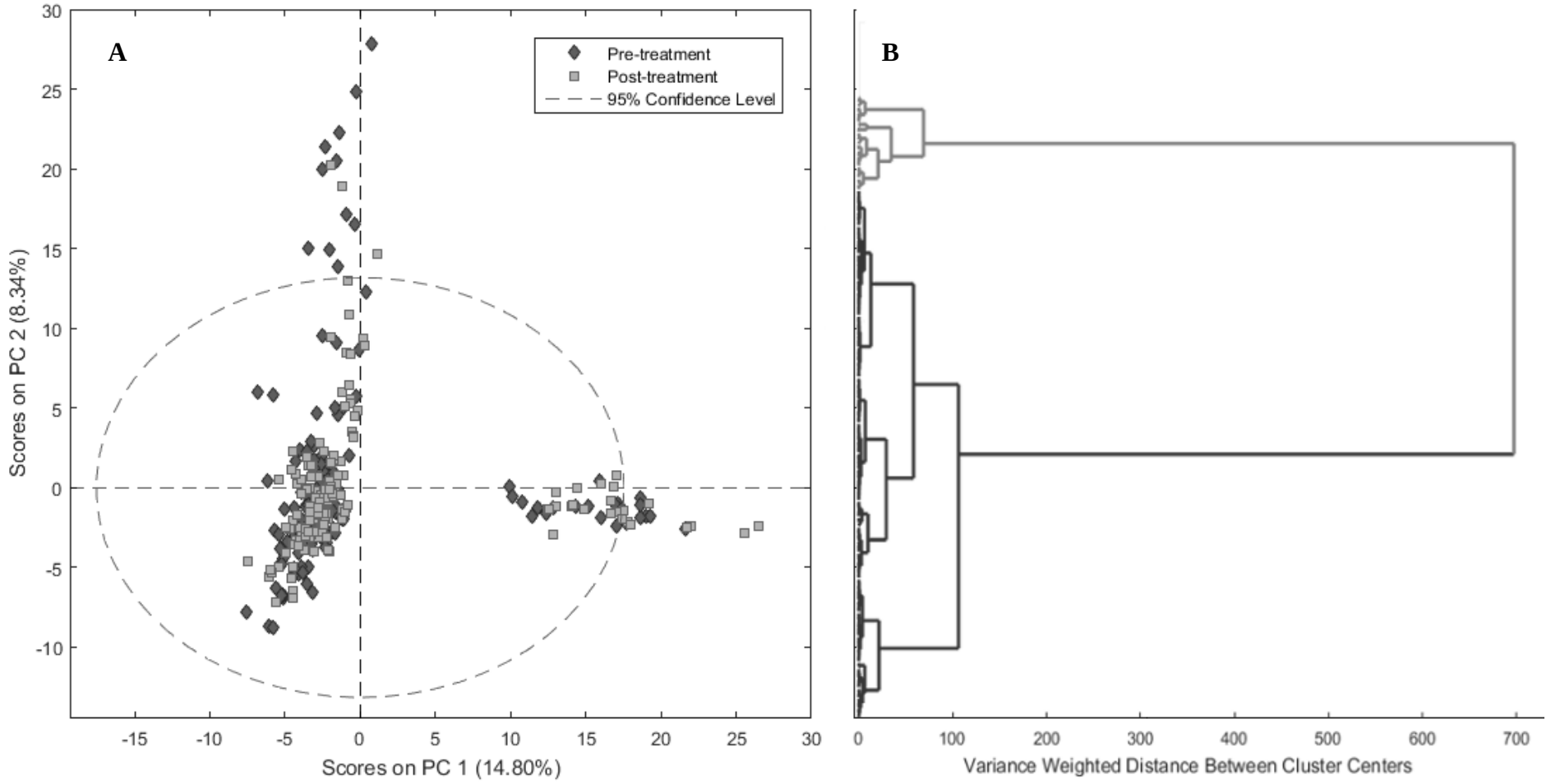
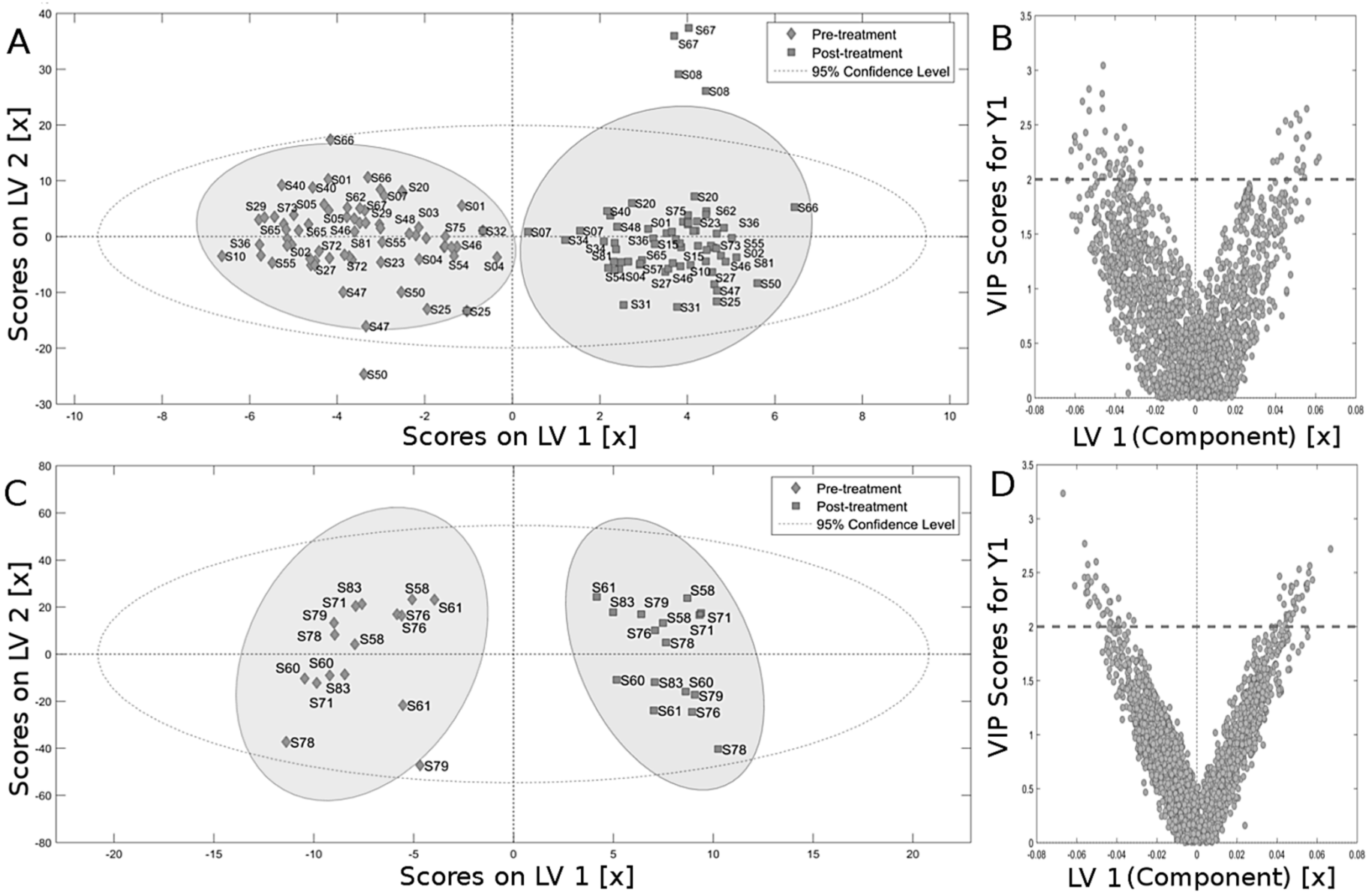

A
Pre-treatment
Post-treatment
95% Confidence Level
Scores on PC 2 (8.34%)
Scores on PC 1 (14.80%)
B
Variance Weighted Distance Between Cluster Centers

FIGURE 4.


A
Pre-treatment
Post-treatment
95% Confidence Level
Scores on LV 2 [x]
Scores on LV 1 [x]
B
VIP Scores for Y1
LV 1 (Component) [x]
C
Pre-treatment
Post-treatment
95% Confidence Level
Scores on LV 2 [x]
Scores on LV 1 [x]
D
VIP Scores for Y1
LV 1 (Component) [x]

Supplemental Information

Impact of a polyphenol-rich extract from *Ascophyllum nodosum* on DNA damage and antioxidant activity in an overweight/obese population

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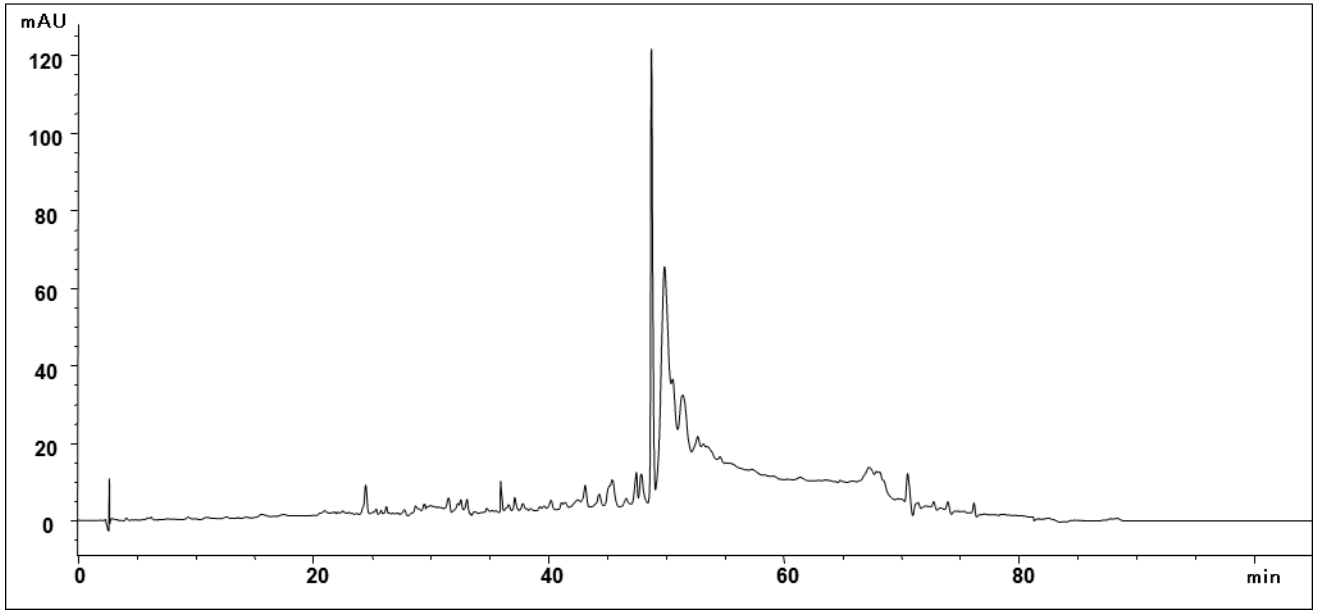
Analysis of the SPE capsule by NP-HPLC and LC-MS analysis (as reported in Corona et al Br J Nutr. 2016 Apr 14;115(7):1240-53.)

NP-HPLC analysis: The phlorotannins in the food-grade SPE used to produce the capsule were analyzed by normal phase HPLC analysis as previously described (Corona G et al, BJN, 2016) using an HPLC 1100 series equipped with LiChrospher Si60-5 column (250 mm × 4.0 mm ID, 5 µm particle size from HICHROM (LISP60-5-250AF), fitted with a guard column LiChrospher Si60-5 from HICHROM (LISP60-5-10C5). The mobile phase contained A: 82 % dichloromethane + 2 % methanol + 2 % acetic acid in water and B: 96 % methanol + 2 % acetic acid in water and was pumped through the column at 1 ml/min. 10 µl of samples were injected and analyzed by the gradient program which were (min/%B): 0/0, 30/17.6,45/30.7, 50/87.8, 60/87.8, 80/0, 105/0 for detection of all compounds. The compounds were detected at a wavelength of 268 nm. All data were analyzed by ChemStation software. The phloroglucinol standard was injected at 0.1-100 µg/ml and phlorotannins in the capsules were analysed as phloroglucinol equivalents.

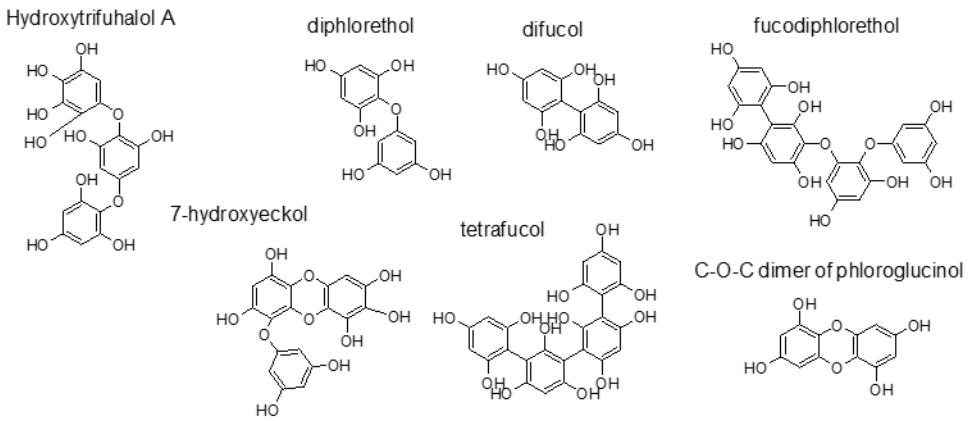
LC-MS analysis: LC-MS analysis was conducted to analyse the food grade seaweed capsule as previously described (Corona G et al, BJN, 2016), and was carried out in the negative ion mode using LC-MS/MS utilizing electrospray ionisation (ESI). Characterization was achieved using LC-MS/MS utilizing electrospray ionisation (ESI). This consisted of an Agilent 1200 HPLC system equipped with a binary pump, degasser, auto-sampler, thermostat, column heater, photodiode array detector and an Agilent 1100 Series LC/MSD Mass Trap Spectrometer. Separation of samples was achieved using a Zorbax SB C18 column (2.1 x 100 mm; 1.8 µm) (Agilent) and HPLC conditions were as follows: injection volume: 1 µL; column temperature: 25 °C; binary mobile system: (A) 0.1 % aqueous formic acid and (B) 0.1 % of formic acid in acetonitrile; flow rate: 0.2 mL/min. A series of linear gradients were used for separation (min/%B): 0/10, 3/10, 15/40, 40/70, 50/70, 65/10. Mass spectrometry was performed in the negative ion mode (scan range, m/z 100-1000 Da; source temperature, 350 °C). All solvents used were LC-MS grade.

The SPE (supplemental figure 1) comprised a wide range of molecular weights (20-70 min), with abundance of very high molecular weight phlorotannins eluting at later retention time (50-70min) in our Normal-Phase method. Using a RP-HPLC separation method coupled to ESI-MS analysis in negative ion mode (supplemental figure 3) we were able to identify some phlorotannin oligomers such as hydroxytrifuhalol A, tetrafucol, fucodiphlorethol, C-O-C dimmer of phloroglucinol, 7-hydroxyeckol, diphloretol and difucol.



Supplemental Figure 1 . Chromatographic separation of phlorotannins contained in the seaweed extract by Normal-Phase HPLC with diode array detection (268nm).

A



B

LC-MS analysis in negative ion mode of the seaweed extract phlorotannins

Peak N	RT (min)	[M-H]⁻ m/z		MS^2 m/z		tentative identification
1	2.7	405	387	191		hydroxytrifuhalol A
2	3.3	497	479	353	205	tetrafucol, fucodiphlorethol
3	5.6	247	203			C-O-C dimer of phloroglucinol
4	10.6	387	369	230		7-hydroxyeckol
5	43.7	249	181	113		diphlorethol / difucol
6	46.2	249	181	113		diphlorethol / difucol

Supplemental figure 2. Characterisation of phlorotannins in the seaweed extract. A: Structures of phlorotannins identified in the seaweed extract: B: Phlorotannins in the seaweed extracts identified by LC-MS analysis in negative ion mode

Extraction /Analysis of Plasma and Urine samples by HPLC DAD.

The HPLC-PDA described below has been removed from the original data due to the limitation of the HPLC DAD approach applied and replaced by analysis as decribed in paper. The data is now only available in supplemental information for context with existing papers on this topic using the same seaweed extract (SPE) .

1) Corona G, Ji Y, Anegboonlap P, Hotchkiss S, Gill C, Yaqoob P, Spencer JP, Rowland I.Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. Br J Nutr. 2016 Apr 14;115(7):1240-53

2).Corona G, Coman MM, Guo Y, Hotchkiss S, Gill C, Yaqoob P, Spencer JP, Rowland I.Effect of simulated gastrointestinal digestion and fermentation on polyphenolic content and bioactivity of brown seaweed phlorotannin-rich extracts. Mol Nutr Food Res. 2017 Nov;61(11). doi: 10.1002/mnfr.201700223. Epub 2017 Aug 29

Extraction of (poly)phenols from urine and blood samples for HPLC DAD

Urine samples were prepared as follows: 10 µl of internal standard solution (resorcinol 200 µg/ml) were added to 250 µl urine. Samples were analysed with and without enzymatic treatment (37°C, 40 min), in the presence of 1500 IU of β-glucuronidase and 50 IU of sulfatases from *Helix pomatia* (Type H-1). 1 ml of methanol acidified with 0.5% acetic acid was added, samples were mixed and centrifuged for 15 min at 16,100 x g at 4°C, supernatants were transferred

to a new tube and dried on a speedvac. Dried samples were resuspended in 125 µl of mobile phase, completely dissolved, centrifuged and transferred to vials for HPLC-DAD.

Plasma samples were prepared as follows: 10 µl of internal standard solution (resorcinol 200 µg/ml) were added to 450 µl of plasma, then 50 µl of 1.2 M acetic acid were added and samples were mixed. Samples were analysed with and without enzymatic treatment (37°C, 40 min) in the presence of 1500 IU of β-glucuronidase and 50 IU of sulfatases from *H. pomatia* (Type H-1). 1 ml of 100% methanol acidified with 0.5% acetic acid was added and samples were centrifuged for 15 min at 16,100 x g at 4°C and supernatants were collected. This step was repeated 3 times, the last occasion with 50% methanol acidified with 0.5% acetic acid, and the supernatants were dried using a speedvac. The pellets were dissolved with 125 µl of mobile phase and transferred to vials for HPLC analysis.

Analysis of seaweed phenolics in blood and urine by HPLC-DAD analysis

Sample analysis was carried out with a Hewlett-Packard 1100 series liquid chromatography system (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector (HP Chem Station Software system). Samples were analysed by reverse-phase HPLC using a Nova-Pak C18 column (4.6 x 250 mm) with 4µm particle size. The temperature of the column was maintained at 30ºC. The mobile phases consisted of a mixture of aqueous methanol 5% in 0.1% hydrochloric acid 5 M (A) and a mixture of aqueous acetonitrile 50 in 0.1% hydrochloric acid 5 M (B) and

were pumped through the column at 0.7 ml/min. The following gradient system was used (min/% B): 0/5, 5/5, 40/50, 55/100, 59,9/100, 60/5, with 10 min post-run for both compound and metabolite detections. The eluent was monitored by photodiode array detection at 280 nm and spectra of products obtained over the 200–600 nm range. Peaks were characterised by their retention time and spectra characteristics. A calibration curve of phloroglucinol was constructed using authentic standard (0.1–100 µg/ml) and in each case was found to be linear with correlation coefficients of 0.995. Metabolites were quantified as phloroglucinol equivalents.

HPLC-DAD analysis of the urine (Supplmental Table 2) and plasma (Supplmental Table 3) samples, with and without glucuronidase/sulfatase treatment, showed the presence of a variety of metabolites absent in the baselines (before the intervention) in both plasma and urine samples. Some metabolite peaks were present in samples with and without enzymatic treatment, and therefore could be assigned to un-conjugated metabolites. Some other metabolite peaks were present only in samples without enzymatic treatment or were only appearing in samples enzymatically treated, and were attributed to conjugated forms (glucuronides and/or sulfates) and their enzymatically released un-conjugated forms. There was substantial inter-individual variation in (poly)phenol s excreted by the participants. In 42 participants, no (poly)phenol s were detected in urine and in the remainder (n=36) the amounts excreted ranged from 0.5 to 11.8 mg/day (mean 8.2 ± 12.1 mg/d). Similarly, there was a large variation in plasma concentrations (42.1 ± 117.0 µg/ml),

with only 14 participants having detectable (poly)phenol s in their plasma, seven of which had no (poly)phenol s detected in their urine samples, in accordance with what was observed after acute consumption. It is worth clarifying that, despite inter-individual variability in polyphenols metabolism being common for many polyphenol classes, in the present case the observed variability could partially be the result of the methodological limitations with the HPLC-DAD analysis.

Supplemental Table 1

RP-HPLC analysis of seaweed metabolites in urine samples. Metabolites were detected in 36 out of 78 participants.

RT (min)	Metabolite	Mean (mg)	Stdev	Volunteers (n)	Enz (-)	Enz (+)
11.9	UM1	11.82	14.14	4	yes	yes
12.8	UM2	3.63	3.03	2	yes	yes
13.1	UM3	6.87	5.01	6	yes	yes
16.3	UM4	3.31	0.03	2	yes	yes
18.6	UM5	3.66	2.07	4	yes	no
21.7	UM6	6.17	3.16	4	yes	yes
24.5	UM7	3.46	2.02	4	no	yes

25.2	UM8	0.47	0.26	2	yes	no
25.9	UM9	2.26	1.18	3	yes	no
28.8	UM10	0.79	0.53	2	no	yes
35.0	UM11	1.57	0.95	3	yes	yes
36.4	UM12	1.02	0.49	4	yes	yes
39.5	UM13	1.18	1.02	4	yes	yes
41.5	UM14	0.95	0.92	4	no	yes
44.2	UM15	0.92	0.74	6	no	yes
48.0	UM16	2.38	2.14	4	yes	no
49.6	UM17	5.42	5.23	5	no	yes
53.8	UM18	0.90	1.17	3	no	yes
Group Mean Total		8.16	12.10	36		

Phenolics in urine were quantified as phloroglucinol equivalents using HPLC and expressed as mg/d in urine. Note; the row "Group Mean Total " represents the average of the sum (total excretion) of metabolites calculated for each individual volunteer.

Supplemental Table 2

RP-HPLC analysis of seaweed metabolites in plasma samples. Metabolites were detected in 14 out of 78 participants.

RT (min)	Metabolite	Mean (µg/ml)	Stdev	Volunteers (n)	Enz (-)	Enz (+)
8.5	PM1	148.12	91.12	2	no	yes
9.1	PM2	0.75	0.31	2	yes	no
11.0	PM3	23.71	12.69	2	yes	no
14.9	PM4	110.85	139.63	2	yes	no
31.0	PM5	2.56	1.54	3	yes	no
37.3	PM6	1.55	0.69	2	yes	yes
43.2	PM7	1.34	0.21	2	no	yes
45.3	PM8	0.64	0.18	2	no	yes
48.0	PM9	0.78	0.16	3	yes	no
58.7	PM10	2.62	0.56	2	yes	yes
Group Mean Total		42.08	117.06	14		

Phenolics in plasma were quantified as phloroglucinol equivalents using HPLC and expressed as µg/ml in plasma.

Note; the row “Group Mean Total ” represents the average of the sum (total excretion) of metabolites calculated for each individual volunteer.

Supplemental Table 3. List of urine metabolites correlated with the chronic intake of seaweed capsules in group 1 formed by 70 participants.

ID	VIP value	Experimental Molecular Weight	Metabolite tentative identification
41	5.8	332.18323	Unknown
67	6.0	332.18323	Unknown
165	5.2	182.99788	Saccharin
292	5.0	182.99797	Saccharin
298	5.1	176.06742	3-hydroxy-2-methoxyglutarate/glycerol-1,2-diacetate/3-isopropylmalate/2,3-dimethyl-3-hydroxyglutaric acid/2-isopropylmalic acid
328	5.6	693.32784	Unknown
372	5.1	182.9979	Saccharin
416	4.9	182.99769	Saccharin
457	4.6	234.01896	Ajoene/4-(2,2´-bithiophen-5-yl)-3-butyn-1-ol
464	5.8	183.0885	Epinephrine/tretraydrohippuric acid/normetanephrine
500	5.4	188.10399	Azelaic acid/butyl ethyl malonate/entre otros
505	4.9	188.10391	Azelaic acid/butyl ethyl malonate/entre otros
556	5.3	183.08848	Epinephrine/tretraydrohippuric acid/normetanephrine
557	4.5	188.10397	Azelaic acid/butyl ethyl malonate/entre otros
602	4.5	174.9926	Unknown
650	6.6	382.16221	Ibuprofen glucuronide/cyclocalopin B
652	3.8	205.98811	Pyrogallol-O-sulfate
800	4.2	205.98816	Pyrogallol-O-sulfate
801	5.2	182.99757	Saccharin
847	3.3	350.1569	Eremopetasin sulfoxide
940	4.2	692.32582	Pisumoside B
956	5.3	372.10485	Dihydroferulic acid-4-O-glucuronide
981	3.6	440.16758	Unknown

1025	4.0	351.09499	Indole-3-acetic-acid-O-glucuronide
1106	5.8	150.01492	Tartalic acid/2,3-dihydroxybutanedioic acid/2-2-trienylfuran
1130	3.7	440.16786	Unknown
1155	3.9	440.16724	Unknown
1213	5.0	542.27235	Cortolone-3-glucuronide
1217	4.3	134.05685	2,3-dihydroxyvaleric acid/deoxyribose/dihydroxy-isovalerate/glycerol acetate
1248	5.2	150.0146	Tartalic acid/2,3-dihydroxybutanedioic acid/2-2-trienylfuran
1262	4.4	134.05696	2,3-dihydroxyvaleric acid/deoxyribose/dihydroxy-isovalerate/glycerol acetate
1269	4.6	134.05687	2,3-dihydroxyvaleric acid/deoxyribose/dihydroxy-isovalerate/glycerol acetate
1303	4.5	134.05689	2,3-dihydroxyvaleric acid/deoxyribose/dihydroxy-isovalerate/glycerol acetate
1306	4.2	440.16771	Unknown
1315	6.1	150.01506	Tartalic acid/2,3-dihydroxybutanedioic acid/2-2-trienylfuran
1352	4.3	486.17277	Hydroxytrifuhaol A-glucuronide
1408	4.3	810.29457	Unknown
1429	4.5	356.11012	Hydroxy-methoxycinnamoyl-b-glucose/1-O-feruloylglucose/veranisatin B
1453	3.8	486.17314	Hydroxytrifuhaol A-glucuronide
1458	4.2	486.1734	Hydroxytrifuhaol A-glucuronide
1472	3.9	205.98794	Pyrogallol-O-sulfate
1483	4.4	486.17327	Hydroxytrifuhaol A-glucuronide
1493	5.0	310.97636	Unknown
1544	4.7	824.274	Unknown
1648	5.3	233.03502	Dopamine sulfate
1665	4.8	435.18873	Unknown
1679	4.7	219.08849	N-deschlorobenzoyl indomethacin/alpha-methoxy-1-H-indole-3-propanoic acid/nigellimine N-oxide/alpha-hydroxy-1H-indole-3-propanoic acid
1693	5.4	251.0786	N-carboxyacetyl-D-phenylalanine/N-phenylacetylaspartic acid/4-hydroxy-3-methoxy-cinnamoylglycine
1711	4.8	233.0349	Dopamine sulfate
1715	4.6	181.07286	Tyrosine
1725	4.2	298.10513	2-Phenylethanol glucuronide

1727	5.2	251.07833	N-carboxyacetyl-D-phenylalanine/N-phenylacetylaspartic acid/4-hydroxy-3-methoxy-cinnamoylglycine
1737	5.0	219.0888	N-deschlorobenzoyl indomethacin/alpha-methoxy-1-H-indole-3-propanoic acid/nigellimine N-oxide/alpha-hydroxy-1H-indole-3-propanoic acid
1751	5.3	414.15204	Ptelatoside A
1778	5.1	251.0785	N-carboxyacetyl-D-phenylalanine/N-phenylacetylaspartic acid/4-hydroxy-3-methoxy-cinnamoylglycine
1799	5.4	842.39386	Unknown
1806	3.5	338.947	Unknown
1828	6.1	364.17303	Unknown
1877	5.2	842.39393	Unknown
1917	5.0	544.28816	Dioxinodehydroeckol glucuronide
1924	4.7	360.1411	Dimethoxyphenyl-ethanediol O-glucoside/2-(4-hydroxy-3,5-dimethoxyphenyl)ethanol 4´-glucoside/ deoxyloganic acid
1935	4.7	223.11987	Cerulenin
1945	4.9	251.0785	N-carboxyacetyl-D-phenylalanine/N-phenylacetylaspartic acid/4-hydroxy-3-methoxy-cinnamoylglycine
1948	4.2	218.02395	tyrosol-sulfate/diphenyl disulfide
1953	4.1	378.05751	Unknown
1961	4.9	400.1728	Corchoionoside B
1970	4.7	223.11986	Cerulenin
1995	5.1	372.10472	Dihydroferulic acid-4-O-glucuronide
2038	5.3	360.14121	Dimethoxyphenyl-ethanediol O-glucoside/2-(4-hydroxy-3,5-dimethoxyphenyl)ethanol 4´-glucoside/ deoxyloganic acid
2040	5.5	526.27719	Unknown
2059	5.4	504.19905	Myricatomentoside I/chlorhexidine
2063	4.4	398.10594	Methyl 3,4-dihydroxy-5-prenylbenzoate-3-glucoside
2072	4.4	372.10487	Dihydroferulic acid-4-O-glucuronide
2080	5.4	324.06294	Sterigmatocystin/greviline A

2081	4.6	251.07794	N-carboxyacetyl-D-phenylalanine/N-phenylacetylaspartic acid/4-hydroxy-3-methoxy-cinnamoylglycine
2185	4.8	280.05956	Unknown
2186	4.8	280.05942	Unknown
2188	4.8	240.12161	Anserine/homocarnosine/balenine
2194	5.4	213.00886	Indoxyl sulfate/carmustine

All predicted formula derived with < 5 ppm mass accuracy data. VIP (variable influence in projection) is a variable that summarizes the importance of X variables to the OPLS-DA model. Variables with VIP values > 2 were the main contributors in the model. ID, identification number. Metabolites were searcher against two data based, (Metlin database; http://metlin.scripps.edu/ and HMDB: http://hmdb.ca). Searching metabolites do not include heavy metal and halogens.

Supplemental Table 4. List of plasma metabolites correlated with the chronic intake of seaweed capsules.

ID	VIP value	Experimental weight	Molecular	Tentative identification
1315	2.1	150.01506		Tartalic acid/2,3-dihydroxybutanedioic acid/2-2-trienylfuran
1450	3.1	188.01341		p-cresol sulfate
246	2.2	195.95296		Unknown
114	2.4	195.953		Unknown
111	2.3	195.95304		Unknown
977	2.3	208.00368		Unknown
2066	2.1	210.03589		Galactaric acid/glucaric acid
797	2.0	213.00848		Indoxyl sulfate/carmustine
1948	2.0	218.02395		tyrosol-sulfate/diphenyl disulfide
1711	2.1	233.0349		Dopamine sulfate
1648	2.3	233.03502		Dopamine sulfate
2097	2.2	233.03511		Dopamine sulfate
587	2.0	236.10413		4-hydroxy-3-methoxy-5-(3-methylbut-2-en-1-yl)benzoic acid/carboxy-ibuprofen/ethyl vanillin isobutyrate
496	2.0	236.10422		4-hydroxy-3-methoxy-5-(3-methylbut-2-en-1-yl)benzoic acid/carboxy-ibuprofen/ethyl vanillin isobutyrate
892	2.1	246.0189		Unknown
2138	2.7	278.03987		Unknown
2186	3.2	280.05942		Unknown
2185	3.3	280.05956		Unknown
706	2.5	293.9301		Unknown
622	2.1	293.9301		Unknown
2119	3.2	324.12077		Unknown
2035	3.0	324.12114		Unknown
588	2.1	326.13624		4-(4´-hydroxyphenyl)2-butanone glucoside/ citrusin C/1-methoxy-3-(4-hydroxyphenyl)-2E-propenal 4´-glucoside

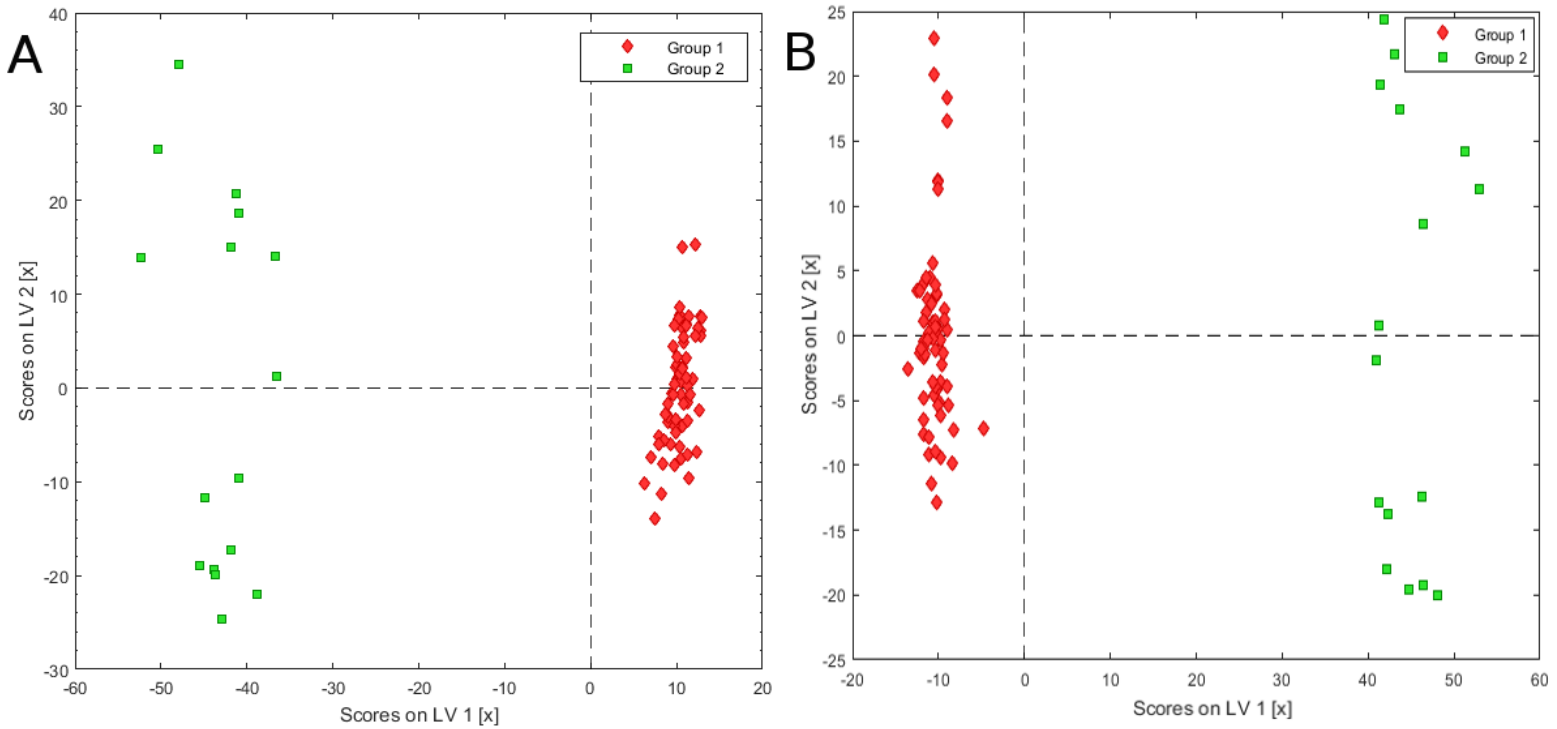
654	2.4	326.13646	4-(4´-hydroxyphenyl)2-butanone glucoside/ citrusin C/1-methoxy-3-(4-hydroxyphenyl)-2E-propenal 4´-glucoside
1293	2.3	330.16753	Diphlorethol sulfate
1356	2.3	330.16795	Diphlorethol sulfate
2163	2.0	336.0294	Unknown
2013	2.0	336.02953	Unknown
1958	2.2	347.14012	Unknown
2068	2.5	358.08919	Dihydrocaffeic acid -O-glucuronide
519	2.6	360.14168	Dimethoxyphenyl-ethanediol O-glucoside/2-(4-hydroxy-3,5-dimethoxyphenyl)ethanol 4´-glucoside/ deoxyloganic acid
430	2.2	360.14198	Dimethoxyphenyl-ethanediol O-glucoside/2-(4-hydroxy-3,5-dimethoxyphenyl)ethanol 4´-glucoside/ deoxyloganic acid
1540	2.3	380.14656	Gibberellin A75/8-oxodiacetoxyscirpenol
1357	2.0	382.16224	Ibuprofen glucuronide/cyclocalopin B
381	2.4	398.15706	Methyl 3,4-dihydroxy-5-prenylbenzoate-3-glucoside
364	2.0	398.15764	Methyl3,4-dihydroxy-5-prenylbenzoate-3-glucoside
234	2.0	412.13594	Methylpicraquassioside A
134	2.3	412.136	Methylpicraquassioside A
214	2.0	412.13618	Methylpicraquassioside A
154	2.0	412.13626	Methylpicraquassioside A
159	2.2	412.13637	Methylpicraquassioside A
164	2.2	412.13649	Methylpicraquassioside A
1751	2.0	414.15204	Ptelatoside A
2134	2.1	440.09245	3,5-dihydroxyphenyl-1-O-(6-O-galloyl-beta-D-glucopyranoside)
1633	2.1	544.21522	Dioxinodehydroeckol glucuronide
351	2.0	796.31532	Unknown
853	2.1	797.31862	Fucophloroethols glucuronide
1182	2.0	169.0363	2-furoylglycine/L-2-3,dihydrodipicolinate
1627	2.4	174.08776	Suberic acid/ 2-propylglutaric acid
753	2.1	174.99243	Unknown

1083	2.1	174.99252	Unknown
602	2.8	174.9926	Unknown
859	2.3	174.9926	Unknown
1023	2.1	174.9926	Unknown
769	2.0	174.99263	Unknown
2094	2.3	189.00848	Lanthionine ketimine/2-aminophenol sulphate
244	2.1	229.00336	Unknown
702	2.0	248.0315	C-O-C dimer of phloroglucinol
1918	2.8	259.99821	Caffeic acid 3-sulfate
887	2.2	261.03016	Unknown
1120	2.8	290.04494	4-hydroxy-5-(hydroxyphenyl)-valeric acid-sulfate
1677	2.1	303.05847	Unknown
1481	2.9	314.09898	2-hydroxyphenylacetic acid-O-glucuronide
1993	2.1	314.09898	2-hydroxyphenylacetic acid-O-glucuronide
293	2.1	327.09512	C-O-C dimer of phloroglucinol-sulfate
839	2.1	328.09828	Difucol/diphlorethol glucuronide
952	2.2	350.02393	Unknown
847	2.0	350.1569	Eremopetasin sulfoxide
1066	2.4	357.10545	HMBOA-GLc (known as 2-O-glucosyl-7-methoxy-1,4-benzoxazin-3-one
625	2.2	357.10548	HMBOA-GLc (known as 2-O-glucosyl-7-methoxy-1,4-benzoxazin-3-one
479	2.1	357.1056	HMBOA-GLc (known as 2-O-glucosyl-7-methoxy-1,4-benzoxazin-3-one
626	2.4	357.10573	HMBOA-GLc (known as 2-O-glucosyl-7-methoxy-1,4-benzoxazin-3-one
1966	2.0	358.08912	Dihydrocaffeic acid-O-glucuronide
1620	2.1	359.06694	Unknown
1351	2.3	362.15735	Unknown
870	2.4	362.15748	Unknown
1792	2.9	368.10989	3-O-feruloylquinic acid/O-caffeoyl-O-methylquinic acid

1758	2.4	370.08925	Feruloyl C-glucuronide/isoferulic acid-3-O-glucuronide/5-hydroxy-6-methoxycoumarin7-glucoside
1683	2.0	370.08926	Feruloyl C-glucuronide/isoferulic acid-3-O-glucuronide/5-hydroxy-6-methoxycoumarin7-glucoside
1788	2.3	370.08955	Feruloyl C-glucuronide/isoferulic acid-3-O-glucuronide/5-hydroxy-6-methoxycoumarin7-glucoside
1682	2.9	372.10469	Dihydroferulic acid-4-O-glucuronide
1757	2.8	372.10469	Dihydroferulic acid-4-O-glucuronide/veranisatin C
1634	2.6	372.10469	Dihydroferulic acid-4-O-glucuronide
1765	2.9	372.10475	Dihydroferulic acid-4-O-glucuronide
1719	2.9	372.10491	Dihydroferulic acid-4-O-glucuronide
1570	2.1	372.10499	Veranisatin C/dihydroferulic acid-glucuronide
1776	2.2	380.16774	3-Methyl-3-butenyl apiosyl-glucoside/prenyl arabinosyl-glucoside/ecabet
1496	2.8	384.05021	Unknown
2017	3.0	384.12615	Unknown
1512	2.0	388.17252	hydroxypropyl-2-methoxyphenoxyl-1,3-propanediol 1 xyloside/jasmonic acid derivative
1941	2.1	388.17283	hydroxypropyl-2-methoxyphenoxyl-1,3-propanediol 1 xyloside/jasmonic acid derivative
1638	2.7	388.17311	hydroxypropyl-2-methoxyphenoxyl-1,3-propanediol 1 xyloside/jasmonic acid derivative
1330	2.7	410.04463	Unknown
1768	2.8	410.0451	Unknown
1341	2.4	446.09862	Unknown
1551	2.3	446.09904	Unknown
1664	2.0	496.23067	Glaucarubin
1781	2.5	509.13896	trans-hydroxycyclohexyl glyburide (Benzenesulfonamides)

All predicted formula derived with < 5 ppm mass accuracy data. VIP (variable influence in projection) is a variable that summarizes the importance of X variables to the OPLS-DA model. Variables with VIP values > 2 were the main

contributors in the model. ID, identification number. Metabolites were searcher against two data based, (Metlin database; http://metlin.scripps.edu/ and HMDB: http://hmdb.ca). Searching metabolites do not include heavy metal and halogens.



Supplemental Figure 3. OPLS-DA scores plots of the urine samples belong to group of participants 1 (70 volunteers-red) and group of subject 2 (8 participants-green) before (A) and after (B) seaweed consumption. LV1: latent variable 1; LV 2: latent variable 2.

Supplemental table 5 Total e

Subject Id	**Pyrogallol/Phloroglucinol-sulfate**
S58	0.000
S61	0.000
S76	0.000
S78	0.000
S60	0.000
S70	0.003
S79	0.000
S83	0.000
S64	0.015
S40	0.047
S27	0.057
S66	0.064
S25	0.040
S65	0.085
S30	0.040
S71	0.000
S10	0.070
S33	0.128
S03	0.085
S59	0.111
S74	0.098
S19	0.156
S43	0.176
S39	0.085
S75	0.065
S01	0.184
S15	0.130
S53	0.125
S41	0.209
S36	0.225
S49	0.327
S82	0.328
S04	0.312
S46	0.347
S57	0.407
S81	0.373
S54	0.232
S35	0.204
S67	0.235
S16	0.447
S05	0.060
S23	0.547
S17	0.508
S08	0.302
S52	0.477
S29	0.407
S56	0.513

S02	0.532
S18	0.635
S38	0.337
S34	0.377
S31	0.531
S21	0.417
S24	0.453
S55	0.596
S22	0.500
S11	0.514
S37	0.287
S48	0.604
S42	0.436
S44	0.846
S63	0.535
S06	0.601
S12	0.366
S13	0.317
S62	0.555
S14	0.640
S20	0.843
S72	0.772
S32	0.682
S73	0.711
S26	0.533
S77	1.089
S07	0.622
S09	0.867
S51	1.158
S47	0.731
S50	1.470

xcretion of identifed seaweed polyphenols in urine (mMc

Pyrogallol/Phloroglucinol-sulfate	Pyrogallol/Phloroglucinol-sulfate
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.004	0.004
0.000	0.000
0.000	0.000
0.016	0.022
0.036	0.004
0.064	0.012
0.091	0.012
0.026	0.019
0.055	0.110
0.062	0.007
0.000	0.000
0.078	0.020
0.259	0.018
0.095	0.017
0.214	0.015
0.148	0.003
0.114	0.026
0.285	0.030
0.119	0.011
0.263	0.023
0.171	0.106
0.105	0.035
0.081	0.093
0.319	0.034
0.313	0.021
0.327	0.090
0.212	0.037
0.348	0.048
0.417	0.014
0.514	0.014
0.570	0.046
0.583	0.045
0.253	0.037
0.493	0.063
0.616	0.074
0.052	0.045
0.320	0.062
0.574	0.108
0.373	0.046
0.511	0.083
0.586	0.056
0.473	0.193

0.662	0.055
0.543	0.074
0.261	0.716
0.580	0.069
0.867	0.085
0.334	0.183
0.573	0.062
0.671	0.094
0.720	0.083
0.693	0.043
0.628	0.058
0.714	0.081
0.340	0.591
0.611	0.157
0.952	0.067
0.673	0.111
0.452	0.042
0.246	0.402
0.944	0.077
0.964	0.037
0.786	0.318
1.026	0.031
1.178	0.105
1.223	0.120
1.039	0.129
0.898	0.284
0.884	0.072
0.977	0.126
0.827	0.167
1.534	0.132
0.984	0.216

ɔles)

Participants
Hydroxytrifuhaol-A-glucuronide
0.000
0.000
0.000
0.000
0.000
0.000
0.000
0.000
0.000
0.029
0.034
0.023
0.062
0.017
0.068
0.000
0.090
0.019
0.094
0.063
0.076
0.073
0.031
0.117
0.093
0.069
0.146
0.156
0.105
0.105
0.076
0.095
0.112
0.095
0.074
0.062
0.123
0.206
0.149
0.064
0.331
0.127
0.070
0.217
0.142
0.120
0.060

0.110
0.065
0.042
0.195
0.041
0.202
0.171
0.103
0.142
0.188
0.216
0.120
0.130
0.089
0.120
0.180
0.375
0.382
0.162
0.165
0.081
0.127
0.086
0.086
0.287
0.078
0.323
0.249
0.181
0.117
0.378

Total mmoles excreted in urine

Hydroxytrifuhaol-A-glucuronide	Hydroxytrifuhaol-A-glucuronide
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.027	0.028
0.030	0.031
0.025	0.028
0.055	0.039
0.022	0.025
0.066	0.064
0.000	0.000
0.080	0.082
0.016	0.015
0.083	0.084
0.050	0.043
0.069	0.069
0.061	0.061
0.032	0.032
0.113	0.106
0.082	0.085
0.063	0.063
0.133	0.133
0.133	0.142
0.088	0.087
0.091	0.086
0.068	0.069
0.097	0.097
0.095	0.101
0.084	0.083
0.070	0.067
0.054	0.056
0.121	0.109
0.199	0.187
0.141	0.135
0.062	0.058
0.299	0.301
0.151	0.115
0.066	0.064
0.193	0.193
0.117	0.102
0.129	0.129
0.115	0.134

0.097	0.093
0.104	0.107
0.081	0.103
0.167	0.153
0.045	0.048
0.193	0.185
0.166	0.155
0.102	0.094
0.126	0.129
0.177	0.165
0.242	0.196
0.117	0.109
0.134	0.126
0.080	0.081
0.098	0.109
0.159	0.163
0.318	0.341
0.335	0.347
0.172	0.147
0.147	0.150
0.076	0.074
0.122	0.116
0.096	0.102
0.080	0.078
0.250	0.229
0.089	0.088
0.286	0.293
0.221	0.226
0.164	0.164
0.108	0.107
0.411	0.343

	(N=78)	
Hydroxytrifuhaol-A-glucuronide	**Dioxinodehydroeckol-glucuronide**	
	0.000	0.000
	0.000	0.000
	0.000	0.001
	0.000	0.000
	0.000	0.000
	0.000	0.000
	0.000	0.000
	0.000	0.000
	0.000	0.001
	0.029	0.000
	0.029	0.000
	0.028	0.000
	0.035	0.001
	0.026	0.000
	0.059	0.000
	0.000	0.000
	0.037	0.000
	0.017	0.000
	0.038	0.001
	0.028	0.000
	0.066	0.000
	0.069	0.000
	0.032	0.000
	0.099	0.001
	0.071	0.000
	0.057	0.000
	0.128	0.001
	0.143	0.000
	0.056	0.001
	0.060	0.000
	0.006	0.000
	0.105	0.000
	0.090	0.000
	0.066	0.000
	0.065	0.000
	0.055	0.001
	0.013	0.000
	0.167	0.000
	0.121	0.000
	0.059	0.000
	0.304	0.000
	0.107	0.001
	0.059	0.000
	0.151	0.000
	0.050	0.000
	0.122	0.000
	0.127	0.000

0.082	0.000
0.113	0.001
0.116	0.000
0.115	0.001
0.047	0.000
0.187	0.001
0.151	0.001
0.094	0.001
0.095	0.000
0.018	0.000
0.194	0.000
0.107	0.000
0.125	0.001
0.076	0.000
0.105	0.000
0.120	0.001
0.182	0.000
0.160	0.002
0.149	0.000
0.116	0.000
0.070	0.000
0.110	0.000
0.108	0.001
0.079	0.000
0.132	0.001
0.089	0.001
0.217	0.001
0.103	0.000
0.162	0.000
0.102	0.001
0.338	0.000

C-O-C dimer of phloroglucinol-sulfat	C-O-C dimer of phloroglucinol
0.000	0.000
0.000	0.001
0.000	0.001
0.000	0.001
0.002	0.000
0.000	0.000
0.000	0.001
0.039	0.006
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.391	0.005
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
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Fucophloroethol glucuronide	Diphlorethol sulfate
0.000	0.000
0.000	0.000
0.000	0.001
0.000	0.001
0.000	0.002
0.000	0.000
0.000	0.009
0.000	0.001
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.004
0.000	0.000
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0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000

Diphlorethol sulfate	Total metabolites (mmoles)
0.000	**0.001**
0.000	**0.001**
0.001	**0.003**
0.001	**0.003**
0.002	**0.006**
0.000	**0.012**
0.008	**0.019**
0.001	**0.047**
0.000	**0.055**
0.000	**0.200**
0.000	**0.258**
0.000	**0.270**
0.000	**0.278**
0.000	**0.341**
0.000	**0.367**
0.004	**0.405**
0.000	**0.459**
0.000	**0.473**
0.000	**0.498**
0.000	**0.525**
0.000	**0.530**
0.000	**0.561**
0.000	**0.618**
0.000	**0.651**
0.000	**0.683**
0.000	**0.714**
0.000	**0.812**
0.000	**0.874**
0.000	**0.899**
0.000	**0.902**
0.000	**0.964**
0.000	**0.972**
0.000	**1.106**
0.000	**1.107**
0.000	**1.212**
0.000	**1.216**
0.000	**1.227**
0.000	**1.254**
0.000	**1.338**
0.000	**1.381**
0.000	**1.394**
0.000	**1.430**
0.000	**1.450**
0.000	**1.477**
0.000	**1.483**
0.000	**1.550**
0.000	**1.616**

0.000	**1.632**
0.000	**1.642**
0.000	**1.657**
0.000	**1.660**
0.000	**1.665**
0.000	**1.701**
0.000	**1.733**
0.000	**1.756**
0.000	**1.796**
0.000	**1.797**
0.000	**1.821**
0.000	**1.852**
0.000	**1.883**
0.000	**1.942**
0.000	**1.986**
0.000	**2.007**
0.000	**2.078**
0.000	**2.192**
0.000	**2.206**
0.000	**2.220**
0.000	**2.249**
0.000	**2.305**
0.000	**2.357**
0.000	**2.377**
0.000	**2.600**
0.000	**2.617**
0.000	**2.699**
0.000	**2.769**
0.000	**2.825**
0.000	**2.832**
0.000	**4.140**

Mean	1.29
Sd	0.88
min	0.001
max	4.140

Low (<.5 mmole	High>2mmole
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
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	1

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	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1
	1

Low (<.5 mmole	High>2mmole
19	16

Subject Id	Total meta	Low (<.5 mmole =1	High>2mmole =2
S58	0.00	1.00	
S61	0.00	1.00	
S76	0.00	1.00	
S78	0.00	1.00	
S60	0.01	1.00	
S70	0.01	1.00	
S79	0.02	1.00	
S83	0.05	1.00	
S64	0.05	1.00	
S40	0.20	1.00	
S27	0.26	1.00	
S66	0.27	1.00	
S25	0.28	1.00	
S65	0.34	1.00	
S30	0.37	1.00	
S71	0.40	1.00	
S10	0.46	1.00	
S33	0.47	1.00	
S03	0.50	1.00	
S59	0.53		
S74	0.53		
S19	0.56		
S43	0.62		
S39	0.65		
S75	0.68		
S01	0.71		
S15	0.81		
S53	0.87		
S41	0.90		
S36	0.90		
S49	0.96		
S82	0.97		
S04	1.11		
S46	1.11		
S57	1.21		
S81	1.22		
S54	1.23		
S35	1.25		
S67	1.34		
S16	1.38		
S05	1.39		
S23	1.43		
S17	1.45		
S08	1.48		
S52	1.48		
S29	1.55		
S56	1.62		
S02	1.63		
S18	1.64		

S38	1.66	
S34	1.66	
S31	1.67	
S21	1.70	
S24	1.73	
S55	1.76	
S22	1.80	
S11	1.80	
S37	1.82	
S48	1.85	
S42	1.88	
S44	1.94	
S63	1.99	
S06	2.01	2.00
S12	2.08	2.00
S13	2.19	2.00
S62	2.21	2.00
S14	2.22	2.00
S20	2.25	2.00
S72	2.31	2.00
S32	2.36	2.00
S73	2.38	2.00
S26	2.60	2.00
S77	2.62	2.00
S07	2.70	2.00
S09	2.77	2.00
S51	2.82	2.00
S47	2.83	2.00
S50	4.14	2.00